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          5  148: Gale Group Trade & Industry
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Health&Wellness DB(SM)_1976-2002/Nov W4
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    (c) 2002 WIPO/Univentio
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00438670

MICROPORATION OF TISSUE FOR DELIVERY OF BIOACTIVE AGENTS

FORMATION DE MICROPORES SUR UN TISSU POUR L'ADMINISTRATION D'AGENTS BIOACTIFS

Patent Applicant/Assignee:

ALTEA TECHNOLOGIES INC,

EPPSTEIN Jonathan A,

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Patent and Priority Information (Country, Number, Date):

Patent: WO 9829134 A2 19980709

Application: WO 97US24127 19971230 (PCT/WO US9724127) Priority Application: US 96778415 19961231

Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

Main International Patent Class: A61K-041/00

Publication Language: English

Fulltext Word Count: 43031

English Abstract

A method of enhancing the permeability of a biological membrane, including the skin or mucosa of an animal or the outer layer of a plant to a permeant is described utilizing microporation of selected depth and optionally one or more of sonic, electromagnetic, mechanical and thermal energy and a chemical enhancer. Microporation is accomplished to form a micropore of selected depth in the biological membrane and the porated site is contacted with the permeant. Additional permeation enhancement measures may be applied to the site to enhance both the flux rate of the permeant into the organism through the micropores as well as into targeted tissues within the organism.

French Abstract

L'invention concerne un procede d'amelioration de la permeabilite d'une membrane biologique, y compris la peau ou les muqueuses d'un animal ou la couche exterieure d'une plante, a un permeant. Le procede fait appel a la formation de micropores d'une profondeur choisie, et eventuellement a une ou plusieurs sources d'energie selectionnees parmi l'energie sonique, electromagnetique, mecanique ou thermique, et a un stimulant chimique. La formation de micropores permet d'obtenir un micropore de profondeur choisie dans la membrane biologique et le site presentant des pores est mis en contact avec le permeant. Des mesures d'amelioration de permeation supplementaires peuvent etre appliquees au site pour ameliorer la vitesse d'ecoulement du permeant dans l'organisme a travers les micropores ainsi que dans des tissus cibles a l'interieur de l'organisme.

Detailed Description

I

MICROPORATION OF TISSUE FOR DELIVERY OF BIOACTIVE AGENTS BACKGROUND OF THE INVENTION

This invention relates generally to the field of transmembrane delivery of drugs or bioactive molecules to an organism. More particularly, this invention relates to a minimally invasive to non-invasive method of increasing the permeability of the skin, mucosal membrane or outer layer of a plant through microporation of this biological membrane, which can be combined with sonic, electromagnetic, and then-nal energy, chemical permeation enhancers, pressure, and the like for selectively enhancing flux rate of bioactive molecules into the organism and, once in the organism, into selected regions of the tissues therein.

The stratum corneum. is chiefly responsible for the well known barrier properties of skin.

Thus, it is this layer that presents the greatest barrier to transdermal flux of drugs or other molecules into the body and of analytes out of the body. The stratum corneum., the outer horny layer of the skin, is a complex structure of compact keratinized cell remnants separated by lipid domains. Compared to the oral or gastric mucosa, the stratum corneum. is much less permeable to molecules either external or internal to the body. The stratum corneurn is formed from keratinocytes, which comprise the majority of epidermal cells, that lose their nuclei and become comeocytes. These dead cells comprise the stratum corneum, which has a thickness of only about 10-30 pm and, as noted above, is a very

resistant waterproof membrane that protects the body from invasion by exterior substances and the outward migration of fluids and dissolved molecules. The stratum comeum is continuously renewed by shedding of corneum cells during desquamination and the formation of new comeum. cells by the keratinization process.

Underlying the stratum corneum is the viable cell layer of the epidermis and the dermis, or connective tissue layer. These layers together make up the skin. Microporation of these underlying layers (the viable cell layer and dermis) has not previously been used but may

enhance transdernial flux. Deep to the dermis are the underlying structures of the body, including fat, muscle, bone, etc.

Microporation of the mucous membrane has not been used previously. The mucous membrane generally lacks a stratum comeum. The most superficial layer is the epithelial layer which consists of numerous layers of viable cells. Deep to the epithelial layer is the lamina propria, or connective tissue layer.

Microporation of plants has been previously limited to select applications in individual cells in laboratory settings. Plant organisms generally have tough outer layers to provide resistance to the elements and disease. Microporation of this tough outer layer of plants enables the delivery of substances usefal for introduction into the plant such as for conferring the desired trait to the plant or for production of a desired substance. For example, a plant may be treated such that each cell of the plant expresses a particular and useful peptide such as a hormone or human insulin.

The flux of a drug or analyte across the biological membrane can be increased by changing either the resistance (the diffusion coefficient) or the driving force (the gradient for diffusion). Flux may be enhanced by the use of so-called penetration or chemical enhancers. Chemical enhancers are well known in the art and a more detailed description will follow.

Another method of increasing the permeability of skin to drugs is iontophoresis.

Iontophoresis involves the application of an external electric field and topical delivery of an ionized form of drug or an un-ionized drug carried with the water flux associated with ion transport (electro-osmosis). While permeation enhancement with iontophoresis has been effective, control of drug delivery and irreversible skin damage are problems associated with the technique.

Sonic energy has also been used to enhance permeability of the skin and synthetic membranes to drugs and other molecules. Ultrasound has been defined as mechanical pressure

waves with frequencies above 20 kHz, H. Lutz et al., Manual of Ultrasound 3-12 (1984). Sonic energy is generated by vibrating a piezoelectric crystal or other electromechanical element by passing an alternating current through the material, R. Brucks et al., 6 Pharm. Res. 697 (1989). The use of sonic energy to increase the permeability of the skin to drug molecules has been termed sonophoresis or phonophoresis.

Although it has been acknowledged that enhancing permeability of the skin should theoretically make it possible to transport molecules from inside the body through the skin to outside the body for collection or monitoring, practicable methods have not been disclosed. U.S.

Patent No. 5,139,023 to Stanley et al. discloses an apparatus and method for noninvasive blood glucose monitoring. In this invention, chemical permeation enhancers are used to increase the permeability of mucosal tissue or skin to glucose. Glucose then passively diffilses through the mucosal tissue or skin and is captured in a receiving medium. The amount of glucose in the receiving medium is measured and correlated to determine the blood glucose level. However, as taught in Stanley et al., this method is much more efficient when used on mucosal tissue, such as buccal tissue, which results in detectable amounts of glucose being collected in the receiving medium after a lag time of about 10-20 minutes. However, the method taught by Stanley et al.

results in an extremely long lag time, ranging from 2 to 24 hours depending on the chemical enhancer composition used, before detectable amounts of glucose can be detected diffusing through human skin (heat-separated epidermis) in vitro. These long lag times may be attributed to the length of time required for the chemical permeation enhancers to passively diffuse through the skin and to enhance the permeability of the barrier stratum corneum, as well as the length of time required for the glucose to passively diffuse out through the skin. Thus, Stanley et al.

clearly does not teach a method for transporting blood glucose or other analytes non-invasively through the skin in a manner that allows for rapid monitoring, as is required for blood glucose monitoring of diabetic patients and for many other body analytes such as blood electrolytes.

While the use of sonic energy for drug delivery is known, results have been largely disappointing in that enhancement of permeability has been relatively low. There is no consensus on the efficacy of sonic energy for increasing drug flux across the skin. While some studies report the success of sonophoresis, J. Davick et al., 68 Phys. Ther. 1672 (1988); J. Griffin et al., 47 Phys. Ther. 594 (1967); J. Griffin & J. Touchstone, 42 Am. J. Phys. Med. 77 (1963); J.

Griffin et al., 44 Am. J. Phys. Med. 20 (1965); D. Levy et al., 83 J. Clin. Invest. 2074); D.

Bommannan et al., 9 Pharm. Res. 559 (1992), others have obtained negative results, H. Benson et al., 69 Phys. Ther. 113 (1988); J. McEInay et al., 20 Br. J. Clin. Pharmacol. 4221 (1985); H.

Pratzel et al., 13 J. Rheumatol. II 22 (1986). Systems in which rodent skin were employed showed the most promising results, whereas systems in which human skin was employed have generally shown disappointing results. It is well known to those skilled in the art that rodent skin is much more permeable than human skin, and consequently the above results do not teach one skilled in the art how to effectively utilize sonophoresis as applied to transdermal delivery and/or monitoring through human skin.

A significant improvement in the use of ultrasonic energy in the monitoring of analytes and also in the delivery of drugs to the body is disclosed and claimed in copending applications Serial No. 08/152,442 filed November 15, 1993, now U.S. Patent No. 5,458,140, and Serial No.

08/152,174 filed December 8, 1993, now U.S. Patent No. 5,445,61 1, both of which are incorporated herein by reference. In these inventions, the transdermal sampling of an analyte or the transdermal delivery of drugs, is accomplished through the use of sonic energy that is modulated in intensity, phase, or frequency or a combination of these parameters coupled with the use of chemical permeation enhancers. Also disclosed is the use of sonic energy, optionally with modulations of frequency, intensity, and/or phase, to controllably push and/or pump molecules through the stratum corneum via perforations introduced by needle puncture, hydraulic jet, laser, electroporation, or other methods.

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The formation of micropores (i.e. microporation) in the stratum corneurn to enhance the delivery of drugs has been the subject of various studies and has resulted in the issuance of patents for such techniques.

Jacques et al., 88 J. Invest. Dermatol. 88-93 (1987), teaches a method of administering a drug by ablating the stratum corneurn of a region of the skin using pulsed laser light of wavelength, pulse length, pulse energy, pulse number, and pulse repetition rate sufficient to ablate the stratum corneurn without significantly damaging the underlying epidermis and then applying the drug to the region of ablation. This work resulted in the issuance of U.S. Patent 4,775,361 to Jacques et al. The ablation of skin through the use of ultraviolet-laser irradiation was earlier reported by Lane et al., 121 Arch. Dermatol. 609-617 (1985). Jacques et al. is restricted to use of few wavelengths of light and expensive lasers.

Tankovich, U.S. Patent No. 5,165,418 (hereinafter, "Tankovich'418"), discloses a method of obtaining a blood sample by irradiating human or animal skin with one or more laser pulses of sufficient energy to cause the vaporization of skin tissue so as to produce a hole in the skin extending through the epidermis and to sever at least one blood vessel, causing a quantity of blood to be expelled through the hole such that it can be collected. Tankovich'418 thus is inadequate for noninvasive or minimally invasive permeabilization of the stratum comeum. such that a drug can be delivered to the body or an analyte from the body can be analyzed.

Tankovich et al., U.S. Patent No. 5,423,803 (hereinafter, "Tankovich'803") discloses a method of laser removal of superficial epidermal skin cells in human skin for cosmetic applications. The method comprises applying a light-absorbing "contaminant" to the outer layers of the epidermis and forcing some of this contaminant into or through the intercellular spaces in the stratum corneum, and illuminating the infiltrated skin with pulses of laser light of sufficient intensity that the amount of energy absorbed by the contaminant will cause the contaminant to explode with sufficient energy to tear off some of the epidermal skin cells. Tankovich'803

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further teaches that there should be high absorption of energy by the contaminant at the wavelength of the laser beam, that the laser beam must be a pulsed beam of less than I gs duration, that the contaminant must be forced into or through the upper layers of the epidermis, and that the contaminant must explode with sufficient energy to tear off epidermal cells upon absorption of the laser energy. This invention also fails to disclose or suggest a method of drug delivery or analyte collection.

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tissue to which the compound was administered but insufficient to cause vaporization of tissue to which the compound had not been administered. The absorbent compound should be soluble in water or serum, such as indocyanine green, chlorophyll, porphyrins, herne-containing compounds, or compounds containing a polyene structure, and power levels are in the range of 50-1 000 W/CM2 or even higher.

Konig et al., DD 2593 5 1, teaches a process for thermal treatment of tumor tissue that comprises depositing a medium in the tumor tissue that absorbs radiation in the red and/or near red infrared spectral region, and irradiating the infiltrated tissue with an appropriate wavelength of laser light. Absorbing media can include methylene blue, reduced porphyrin or its aggregates, and phthalocyanine blue. Methylene blue, which strongly absorbs at 600-700 nm, and a krypton laser emitting at 647 and 676 run are exemplified. The power level should be at least 200 MW/CM2.

It has been shown that by stripping the stratum corneurn from a small area of the skin with repeated application and removal of cellophane tape to the same location one can easily collect arbitrary quantities of interstitial fluid, which can then be assayed for a number

of analytes SUBSTITUTE SHEET (RULE 26)
of interest. Similarly, the'*tape*-*stripped*'skin has also been shown to be permeable to the transdermal delivery of compounds into the body. Unfortunately, '*tape*-*stripping*'leaves a open sore which takes weeks to heal, and for this, as well as other reasons, is not considered as an acceptable practice for enhancing transcutaneous transport in wide applications.

As discussed above, it has been shown that pulsed lasers, such as the excimer laser operating at 193 mn, the erbium laser operating near 2.9 PM or the C02 laser operating at 10.2 pm, can be used to effectively ablate small holes in the human stratum corneum. These laser ablation techniques offer the potential for a selective and potentially non-traumatic method for opening a delivery and/or sampling hole through the stratum corneum. However, due to the prohibitively high costs associated with these light sources, there have been no commercial products developed based on this concept. The presently disclosed invention, by defining a method for directly conducting thermal energy into or through the biological membrane with very tightly defined spatial and temporal resolution, makes it possible to produce the desired micro-ablation of the biological membrane very low cost energy sources.

In view of the foregoing problems and/or deficiencies, the development of a method for safely enhancing the permeability of the biological membrane for minimally invasive or noninvasive monitoring of body analytes in a more rapid time frame would be a significant advancement in the art. It would be another significant advancement in the art to provide a method of minimally invasively or non-invasively enhancing the transmembrane flux rate of a drug into a selected area of an organism. u!
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00438670
MICROPORATION OF TISSUE FOR DELIVERY OF BIOACTIVE AGENTS
FORMATION DE MICROPORES SUR UN TISSU POUR L'ADMINISTRATION D'AGENTS BIOACTIFS
Patent Applicant/Assignee:
ALTEA TECHNOLOGIES INC,
EPPSTEIN Jonathan A,
Inventor(s):
EPPSTEIN Jonathan A,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9829134 A2 19980709
Application: WO 97US24127 19971230 (PCT/WO US9724127) Priority Application: US 96778415 19961231
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 43031

English Abstract
A method of enhancing the permeability of a biological membrane, including the skin or mucosa of an animal or the outer layer of a plant to a permeant is described utilizing microporation of selected depth and optionally one or more of sonic, electromagnetic, mechanical and thermal energy and a chemical enhancer. Microporation is accomplished to form a micropore of selected depth in the biological membrane and the porated site is contacted with the permeant. Additional permeation enhancement measures may be applied to the site to enhance both the flux rate of the permeant into the organism through the micropores as well as into targeted tissues within the organism.

French Abstract
L'invention concerne un procede d'amelioration de la permeabilite d'une membrane biologique, y compris la peau ou les muqueuses d'un animal ou la couche exterieure d'une plante, a un permeant. Le procede fait appel a la formation de micropores d'une profondeur choisie, et eventuellement a une ou plusieurs sources d'energie selectionnees parmi l'energie sonique, electromagnetique, mecanique ou thermique, et a un stimulant chimique. La formation de micropores permet d'obtenir un micropore de profondeur choisie dans la membrane biologique et le site presentant des pores est mis en contact avec le permeant. Des mesures d'amelioration de permeation supplementaires peuvent etre appliquees au site pour ameliorer la vitesse d'ecoulement du permeant dans l'organisme a travers les micropores ainsi que dans des tissus cibles a l'interieur de l'organisme.
Fulltext Availability:
Detailed Description
Claims

Detailed Description
... easily collect arbitrary quantities of interstitial fluid, which can then be assayed for a number of analytes
SUBSTITUTE SHEET (RULE 26)
of interest. Similarly, the'*tape*-*stripped*'skin has also been shown to be permeable to the transdermal delivery of compounds into the body. Unfortunately, '*tape*-*stripping*'leaves a open sore which takes weeks to heal, and for this, as well as other reasons, is not considered as an acceptable practice for...said permeant comprises a nucleic acid. More specifically, the

invention includes the method wherein said nucleic acid comprises DNA or wherein the nucleic acid comprises *RNA*.

The invention further includes the method of enhancing the transmembrane flux rate of a permeant wherein the micropore in the biological membrane extends into a...invention, both ionized and nonionized drugs may be delivered, as can drugs of either high, medium or low molecular weight.

Delivery of DNA and/or *RNA* can be used to achieve expression of a polypeptide, stimulate an immune response, or to inhibit expression of a polypeptide through the use of an 11antisense" nucleic acid, especially an antisense *RNA*. The term "polypeptide" is used herein SUBSTITUTE SHEET (RULE 26)

without any particular intended size limitation, unless a particular size is otherwise stated, and includes...

...be considered limiting: the only limitation to the peptide or protein drug that may be expressed is one of functionality. Delivery of DNA and/or *RNA* is useful in gene therapy, vaccination, and any therapeutic situation in which a nucleic acid or a polypeptide should be administered in vivo. E.g...

...Another illustrative embodiment of the invention is a method for obtaining transitory expression of a polypeptide comprising porating the biological membrane and then delivering an *RNA* or DNA encoding the ...through the pores of the biological membrane, whereby cells of the tissue (e.g., the skin, mucous membrane, capillaries, or underlying tissue) take up the *RNA* or DNA and produce the polypeptide for less than about 20 days, usually less than about 10 days, and often less than about 3-5 days. The cells which take

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up the *RNA* or DNA could include the cells of the biological membrane, the underlying tissue or other target tissue reached by way of the capillaries.

The DNA and/or *RNA* can be naked nucleic acid optionally in a carrier or vehicle, and/or can be contained within microspheres, liposomes and/or associated with transfection-facilitating...

...or is nonintegrating, in a plasmid, or as a naked polynucleotide. The nucleic acid can encode a polypeptide, or alternatively can code for an antisense *RNA*, for example for inhibiting translation of a selected polypeptide in a cell. When the nucleic acid is DNA, it can be a DNA sequence that...

...the invention, the method provides for immunizing an individual, wherein such individual can be a human or an animal, comprising delivering a DNA and/or *RNA* to the individual wherein the DNA and/or *RNA* codes for an immunogenic translation product that elicits an immune response against the immunogen. The method can be used to elicit a humoral immune response...

...by those of ordinary skill in the art with a virtually unlimited number of cDNAs. Such plasmids can advantageously comprise a promoter for a selected *RNA* polymerase, followed by a Yuntranslated region, a Tuntranslated ...insert any selected cDNA coding for a polypeptide of interest. Although good results can be obtained with pSP64T when linearized and then transcribed with SP6 *RNA* polymerase, it is preferable to use the Xenopus-globin flanking sequences of pSP64T with the phage T7 *RNA* polymerase. This is accomplished by purifying an approximately 150 bp HindHI/EcoRI fragment from pSP64T and inserting it into a linearized approximately 2.9 kb...multiple cloning site (MCS) of unique XhoI, XbaI, SmaI, 1 5 SacI, and Bam11I restriction sites. DNA fragments cloned into the MCS are transcribed into *RNA* from the SV40 late promoter and are translated from the first ATG codon in the cloned fragments. Transcripts of cloned DNA are spliced and polyadenylated...vaccine is prepared as a solid, liquid, suspension, or gel as required. This formulation could include any one or combination of peptides, proteins, carbohydrates, DNA, *RNA*, entire microorganisms, adjuvants, carriers and the like. A selected site of an individual is porated (skin or mucous membrane) according to the procedures described above...

Claim

... acid.

49 The method of Claim 48 wherein said nucleic acid comprises a DNA.

50 The method of Claim 48 wherein said nucleic acid comprises *RNA*.

51 The method of claim 1, wherein the inicropore in the biological membrane extends into a portion of the outer layer of the biological membrane...

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00432861
COMPOUND DELIVERY USING IMPULSE TRANSIENTS
APPORT DE COMPOSES AU MOYEN DE PHENOMENES TRANSITOIRES IMPULSIONNELS Patent
Applicant/Assignee:
THE GENERAL HOSPITAL CORPORATION,
Inventor(s):
KOLLIAS Nikiforos,
DOUKAS Apostolos G,
FLOTTE Thomas J,
McAULIFFE Daniel J,
LEE Shun,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9823325 A1 19980604
Application: WO 97US22166 19971126 (PCT/WO

US9722166) Priority Application: US 9631882 19961127 Designated States: CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE Publication Language: English Fulltext Word Count: 9077

English Abstract

A method for delivering compounds through epithelial cell layers using impulse transients is described. The method involves applying a compound to, e.g., the stratum corneum, of a patient and then inducing impulse transients to create transient increases in the permeability of epithelial tissue, thereby facilitating delivery of the compound across the epithelial cell layer.

French Abstract

L'invention concerne un procede d'apport de composes, a travers les couches de cellules epitheliales, faisant appel a des phenomenes transitoires. Le procede consiste a appliquer un compose sur, par exemple, la couche cornee de la peau d'un patient, puis a provoquer des phenomenes transitoires impulsionnels de maniere a augmenter, de facon transitoire, la permeabilite de l'epithelium, ce qui facilite l'apport du compose a travers la couche de cellules epitheliales.

Fulltext Availability:
Detailed Description

Detailed Description
... to the impulse transient
(laser stress wave 11LSW11) in the presence of 40 kDa dextran (after LSW) and after the stratum corneum was
removed by *tape* *stripping* ("SC removed") (Fig. 5A; and of lo the emission spectra of the exposed (+LSW) and control LSW) sites after subtraction of baseline fluorescence
(Fig, 5B...used to transport many different types of compounds. Thus, chemotherapeutic agents such as cisplatin, polypeptides, such as antibodies, nucleic
3o acids,, such as oligonucleotides, DNA, *RNA*, and plasmids, local anesthetics? such as lidocaine and benzocaine, and photosensitizers, such as benzoporpherene derivative
monoacid ring A (BPD-MA), all can be delivered through...as the broken line labeled
"after LSW,11 for laser stress wave), and (3) after the stratum corneum of the exposed site was removed by *tape* *stripping* (shown as the solid line marked "SC removed"), *Tape* *stripping* was performed to eliminate the fluorescence from the probe molecules located in the
stratum corneum, Thus, the fluorescence signal in the *tape* *stripping* experiment represented only the probe molecules located in the viable epidermis and dermis,
Twenty *tape* *strippings* were sufficient to remove the 35 stratum corneum (Wells, Br. J, Dermatol, 108:87-91,
- 24
1957). The data shown in Fig, ...to procedures that remove the stratum corneum.

Fig, 5B shows the comparative fluorescence of sites exposed to the LSW (+LSW) and control sites (-LSW) after *tape* *stripping* and after the baseline fluorescence has been subtracted. The site subjected to an impulse
transient showed over two-fold higher rhodamine associated fluorescence than the...

...Latex fluorescent particles of 10 nm diameter were also delivered through the stratum corneum using impulse transients, Fig, 6 shows the fluorescence emission
spectra after *tape* *stripping* of a site exposed to a impulse transient using latex particles as the fluorescent probe, The fluorescence emission of the control site under identical conditions...

...site (solid line), 2% SLS was used as the coupling medium; at the other, no SLS was added (dashed line).

The two skin sites were *tape* *stripped* prior to generating 2 5 the two spectra shown in Fig. 7
A comparison of the spectra shown in Fig. 7 reveals that the fluorescence...

3/3,AB,KWIC/3
DIALOG(R)File 349:PCT FULLTEXT
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00412088
INFECTIOUS PAPILLOMAVIRUS PSEUDOVIRAL PARTICLES
PARTICULES PSEUDOVIRALES DE PAPILLOMAVIRUS INFECTIEUX
Patent Applicant/Assignee:
THE GOVERNMENT OF THE UNITED STATES OF AMERICA represented by THE SECRETARY DEPARTMENT OF HEALTH AND HUMAN SERVICES,
LOWY Douglas R,
SCHILLER John T,
RODEN Richard B,
Inventor(s):
LOWY Douglas R,
SCHILLER John T,
RODEN Richard B,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9802548 A2 19980122
Application: WO 97US12115 19970714 (PCT/WO US9712115) Priority Application: US 9622104 19960717 Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN

MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN GH KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG Publication Language: English
Fulltext Word Count: 19858

English Abstract

The invention provides an infectious papillomavirus pseudoviral particle useful in gene transfer comprising: (a) a papillomavirus vector DNA which comprises an E2 binding site and an expression cassette comprising a gene and a sequence controlling expression of said gene; and (b) a papillomavirus capsid which comprises L1 and L2 strutural proteins, such that said capsid encapsidates said vector DNA, wherein said gene is derived from a first biological species and said L1 structural protein is derived from a second biological species and said first biological species is different from said second biological species.

French Abstract

L'invention a pour objet une particule pseudovirale de papillomavirus infectieux particulierement utile dans le transfert des genes. Cette particule comprend (a) un ADN vecteur de papillomavirus qui comprend un site de liaison E2 et une cassette d'expression comprend un gene et une expression de commande de sequence de ce gene; et (b) un capside de papillomavirus qui comprend des proteines structurelles L1 et L2, de telle sorte que le capside encapside l'ADN vecteur, et ce gene est derive d'une premiere espece biologique. La proteine structurelle L1 est derivee d'une deuxieme espece biologique et la premiere espece biologique est differente de la deuxieme espece biologique.

Fulltext Availability:
Detailed Description

Detailed Description
... related genital HPV types.

BPV Ll expressed from recombinant SFV in mammalian cells binds L2 and assembles into VLPs.

SFV is a simple positive strand *RNA* virus. The pSFV-1 expression vector contains the gene for the SFV *RNA* replicase, the inserted gene and a cis acting virion packaging signal. In vitro synthesized *RNA* from this vector is co-transfected with a helper vector (pHelper-2) *RNA* that encodes the SFV structural genes. Upon transfection, the replicase is translated and initiates successive rounds of *RNA* replication and translation, thereby amplifying the viral RNAs. Translation of the helper *RNA* leads to production of the SFV virion proteins and encapsidation of the expression vector *RNA*, but not that of the helper, which lacks the packaging signal. Therefore, the high titer virus generated is defective because it does not encode the SFV virion proteins. Upon infection of susceptible cells (eg., BHK-21 or BPHE-1), the replicase again amplifies the infecting *RNA*. Amplification of subgenomic RNAs encoding 5 the cloned gene leads to high level expression of the encoded protein.

Defective BPV1 Li and BPV1 L2 recombinant Semliki Forest Viruses (SFV-BL1 and SFV-BL2) were generated by co-transfecting BHK-21 cells with in vitro transcribed Helper-2 *RNA* (Life Technologies) (Berglund, P., et al., 1993, BioTechnology 11, 916-920) and a recombinant pSFV-1 *RNA* encoding the BPV1 Ll or BPV1 L2 gene. ...763-68). Because their BPV preparations contained infectious vaccinia virus, which is cytotoxic for many cell types, including C127, they used transient expression of viral *RNA* as their marker for infectivity. One notable difference between the results reported in that study and those obtained here was that their infectivity marker was...which do not contain the papillomavirus genome) were infected with both the L2-SFV recombinant and a SFV recombinant expressing the fulllength E2. Since the *RNA* for E2 was produced entirely by the SFV *RNA*-dependent polymerase in the cytoplasm, production of the alternative E2 mRNAs was precluded. As expected, only the 48 kD form was detected on Western blots...upstream regulatory region (URR) of all papillornaviruses (Turek, L., 1994, Adv. Virus Res. 44, 305-356). The E2 protein has been shown to stimulate viral *RNA* synthesis and viral DNA synthesis. Both of these activities depend upon the binding of E2 to its cognate binding sites (E2BS) in the URR, while ...DNA binding protein brings viral DNA to the developing virion, may apply to other viruses as well. In most instances, the DNA binding protein (or *RNA* binding protein for *RNA* viruses) will be virally encoded. However, there could be viruses in which the DNA binding protein is cell encoded. See Example 6.

Based on the...a gene gun) or topical application (for example, with a gene cream) which may or 1 0 may not require exposure of underlying cells by *tape* *stripping* or penetration enhancers.

It will be appreciated that the actual preferred ...plasmid were linearized using Spe I (or Nru I for pSFV Nrul based clones).

The DNAs were phenolichloroform extracted and ethanol precipitated. To generate SFV *RNA*, 1 /ig of each linearized pSFV-1 clone and 1 /jg of pHelper-2 were resuspended in 100 jil reactions containing 1 mM ATP, 1 mM CTP, 1 mM UTP, 0.5 mM GTP, 1 mM *RNA* capping analog m7G(5')ppp(5')G, 5 mM DTT, 10OU human placental RNase inhibitor, 75U SP6 *RNA* polyrnerase in 1x SP6 reaction buffer. The reaction mixtures were incubated for 1h at 37'C and 2.5 jA was analyzed on a C.7% agarose gel to assess the integrity of the SFV RNAs. The remaining *RNA* was diluted in 1 ml

OptiMEM medium, mixed with 100 /jI of Lipofactin in 1 ml of OptiMEM and incubated for 15 min at ambient temperature. BHK-21 cells in a T-75 tissue culture flask were washed and covered with 2 ml of OptiMEM. The *RNA*/Lipofectin mix was added, and the cells were incubated for 4h at 370C. The cells were washed once and maintained for 24h in 13 ml...

3/3,AB,KWIC/4
DIALOG(R)File 349:PCT FULLTEXT

00357652
NUCLEOTIDE ANALOGUES FOR TOPICAL TREATMENT OF PROLIFERATIVE DISEASE OF THE SKIN
ANALOGUES DE NUCLEOTIDES UTILES POUR LE TRAITEMENT TOPIQUE DE MALADIES PROLIFERATIVES DE LA PEAU
Patent Applicant/Assignee:
HOSTETLER Karl Y,
Inventor(s):
HOSTETLER Karl Y,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9640166 A1 19961219
Application: WO 96US10084 19960606 (PCT/WO US9610084) Priority Application: US 95485025 19950607
Designated States: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 12368
English Abstract
Pharmaceutical compositions containing mono-, di-, and triphosphate esters of antiproliferative nucleoside analogues, DNA chain-terminating dideoxynucleoside analogues and other nucleoside analogues for the topical treatment of hyperproliferative diseases of the skin, including psoriasis. The useful phosphate esters of the nucleoside analogues include phosphoramidates and phosphothiorates, as well as polyphosphates having C and S bridging atoms.

French Abstract
Les compositions pharmaceutiques decrites contiennent des esters de monophosphate, de diphosphate et de triphosphate d'analogues de nucleosides antiproliferatifs, des analogues de didesoxynucleosides de fin de chaine d'ADN et d'autres analogues de nucleosides, et sont utiles pour le traitement topique de maladies hyperproliferatives de la peau, y compris le psoriasis. Les esters de phosphate utiles des analogues de nucleosides comprennent les phosphoramidates et les phosphothiorates, ainsi que les polyphosphates possedant des atomes de pontage C et S.
Fulltext Availability:
Detailed Description
Detailed Description
... membrane of hyperproliferative skin cells and reduce the rate of cell division by inhibiting various enzymatic steps in the biosynthesis of purines and pyrimidines, nucleotides, *RNA* and DNA. Also, the compounds 2-chlorodeoxyadenosine phosphate and 2-chloro-2'-fluoroaradeoxyadenosine phosphate are particularly useful in treating the inflammatory component of psoriasis because...formation of polyamines which are generally elevated in hyperplasia including epidermal hyperplasia associated with psoriasis. Increased ODC activity can also be induced by stratum corneum *tape* *stripping* (Lesiewicz, et al., in Models in Dermatology, vol. 2, pp. 112-116 (H.I. Maibach & N.J. Lowe, eds.), 1985) or by application of a...

3/3,AB,KWIC/5
DIALOG(R)File 349:PCT FULLTEXT

00282505
RECOMBINANT STRATUM CORNEUM CHYMOTRYPTIC ENZYME (SCCE)
ENZYME CHYMOTRYPTIQUE DE LA COUCHE CORNEE (ECCC) RECOMBINEE Patent Applicant/Assignee:
SYMBICOM AKTIEBOLAG,
EGELRUD Torbjorn,
HANSSON Lennart,
Inventor(s):
EGELRUD Torbjorn,
HANSSON Lennart,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9500651 A1 19950105
Application: WO 94IB166 19940620 (PCT/WO IB9400166) Priority Application: DK 72593 19930618
Designated States: AM AU BB BG BR BY CA CN CZ CZ DE DE DK DK FI FI GE HU JP KE KG KP KR KZ LK LV MD MG MN MW NO NZ PL RO RU SD SI SK SK TJ TT UA US UZ VN AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 28346
English Abstract
The present invention relates to a polypeptide having the amino acid sequence SEQ ID NO:2 or an analogue or variant thereof having SCCE activity as defined in the present application, such as a polypeptide having a subsequence of the amino acid sequence SEQ ID NO:2. Furthermore, the present invention relates to nucleotide

sequences encoding polypeptides having SCCE activity as well as to expression systems, expression vectors, plasmids and non-human organisms comprising said nucleotide sequences. Important aspects of the present invention relate to pharmaceutical, cosmetic and skin care compositions comprising a polypeptide having SCCE activity, and the use of a polypeptide having SCCE activity for the treatment or prophylaxis of various diseases such as acne, xeroderma or other hyperkeratotic conditions such as callosities and keratosis pilaris as well as the various ichthyoses, psoriasis and other inflammatory skin diseases such as eczemas. Moreover, the present invention relates to the use of a compound which has an inhibitory effect on the enzymatic activity of native SCCE for the manufacture of a pharmaceutical composition for treatment or prophylaxis of autoimmune pemphigus diseases or acantholytic diseases such as familiar pemphigus and Darier's disease.

French Abstract

La presente invention se rapporte a un polypeptide possedant la sequence d'acides amines SEQ ID NO:2 ou un analogue ou une variante de celle-ci ayant l'activite de ECCC telle que definie dans la presente application, tel qu'un polypeptide ayant une sous-sequence de la sequence d'acides amines SEQ ID NO:2. De plus, la presente invention concerne des sequences nucleotidiques codant des polypeptides ayant l'activite de ECCC ainsi que des systemes d'expression, des vecteurs d'expression, des plasmides et des organismes non humains comprenant ces sequences nucleotidiques. Les aspects importants de la presente invention se rapportent a des compositions pharmaceutiques, cosmetiques et dermatologiques comprenant un polypeptide ayant l'activite de ECCC, et a l'utilisation d'un polypeptide ayant l'activite de ECCC dans le traitement ou la prophylaxie de diverses affections telles que l'acne, la xerodermie ou autres etats hyperkeratosiques, tels que les callosites et la keratose pilaire simple, ainsi que les diverses ichtyoses, psoriasis et autres maladies cutanees inflammatoires, telles que les eczemas. La presente invention se rapporte egalement a l'utilisation d'un compose qui a un effet inhibiteur sur l'activite enzymatique de l'ECCC native, destine a la fabrication d'une composition pharmaceutique s'utilisant dans le traitement ou la prophylaxie des maladies autoimmunes du pemphigus ou des maladies acantholytiques, telles que la maladie de Hailey-Hailey et la maladie de Darier.

Fulltext Availability:

Detailed Description

Detailed Description

... compared with the amino acid

sequence shown in SEQ ID NO:2 may be deduced from a nucleo tide sequence such as a DNA or *RNA* sequence, e.g. obtained by hybridization as defined in the following, or may be obtained by conventional amino acid sequencing methods. The degree of homology...in vitro or recombinant form.

In one embodiment of the invention, detection and/or quanti tation of SCCE polypeptide mRNA may be obtained by extracting *RNA* from cells or tissues and converting it into cDNA for subsequent use in the polymerase chain reaction (PCR). The PCR primer(s) may be synthesized...regulating the activity exerted by an SCCE poly

peptide. This activity may have important implication for various disease conditions as described above.

A DNA or *RNA* fragment complementary to at least part of the mRNA corresponding to the polypeptide of invention or an analogue thereof may be effective in arresting the...length human SCCE cDNA cloned into pUC19. For details

see Example 6.

Fig. 16.

Northern blots with mRNA prepared from human epidermis.

Poly-T-purified *RNA* corresponding to approximately 100 g of total *RNA* was applied in each lane. 1: Hybridization carried out with a probe prepared from a 1070 bp Hinc 2/Hinc 2 frag ment of SCCE...

...SCCE in mammalian cells.

Fig* 19e

Figure 19 shows analysis of expression of the recombinant human SCCE gene of pS507 in mammalian cells.

Lane 1: *RNA* from C127 cells.

Lane 2: *RNA* from an isolated clone, 1:24, of C127 cells transfected with pS507.

Lane 3: *RNA* from a population mixture of C127 clones transfected with pS507.

Lanes 4and 5: *RNA* from C127 cells transfected with an ex pression vector, pS147, which is identical to pS507 except that it lacks the SCCE cDNA se quence. Size...into EcoRI linearized pUC19. The resulting plasmid was completely sequenced and designated pS501.

EXAMPLE 7

Detection of SCCE MRNA in human epidermis

Preparation of total *RNA* from human epidermis

This was carried out ...solu

tion D (Chomczynski and Sacchi, 1987) and homogenized with a glass homogenizer. The protocol described by

Chomczynski and Sacchi was then followed, Pelleted total *RNA* was stored at -200C in 7091; ethanol until further analyzed.

Preparation of messenger *RNA*
Five hundred micrograms of total epidermis *RNA* were processed with the Poly A Tract-kit (Promega) according to the
instructions of the supplier.

RNA-eletrophoresis and blotting
The agarose gels (1.41i) were prepared with 0,66M formaldehyde in I x MOPS buffer and 0.6 g/ml ethidium bromide (Sigma, St.
Louis, MO), MRNA corresponding to 100 gg of total *RNA* was dissolved in *RNA*-sample buffer (50%-formamide, 2.2M form aldehyde, 3!k Ficoll, 1 x MOPS) and heated at 600C for 5 minutes before application. *RNA*-markers (BRL, Gaithersburg, MD) were similarly treated. After the electrophoresis the gels were soaked in distilled water for 5 minutes followed by 50 mM NaOH...

...for 1 hour in 10 x SSC. The
membranes were then washed in 3 x SCC, dried overnight, and baked for 2 hours at 800C. *RNA* was visualized on the membranes under UV-light.

cDNA-probes
The plasmid pS501, prepared as described in Example 6, was digested with HincII and BglII...resistant cell clones were identified and isolated from the master plates and passaged for subsequent analysis.

To analyze the expression of recombinant SCCE genes total *RNA* was prepared from the isolated cell lines. Total *RNA* was prepared from C127 cells and separated on a It formaldehyde
agarose gel, transferred to nitrocellulose membrane and hybridized to a 32P-labelled SCCE probe...

...IgG (Sigma, St. Louis, MO) was
used for enzyme labelling. The results are shown in Fig. 20.
To analyze the expression of recombinant SCCE, total *RNA* was prepared from C127 cells transfected with the expression vector pS507. As control samples, total *RNA* was prepared from both non-transfected C127 cells and from C127 cells trans fected with expression vector pS147. The vector pSI47 is similar to pS507 except that it contains the cDNA for human bile salt-stimulated lipase (Nilsson et al., 1990) instead for the human SCCE cDNA. *RNA* was prepared according to Ausubel et al. (1992). Northern blot experiments and hybri dization with 32p
labelled SCCE cDNA showed that recombinant
SCCE mRNA of...enhance the activity of
SCCE can be prepared.

EXAMPLE 12
Desmosome digestion activity of recombinant SCCE
Corneocytes containing intact desmosomes were removed from skin by *tape* *stripping* to the deeper layers of stratum corneum, and the squames were detached with hexane and dried down in aliquots. Corneocytes aliquots (3 mg) were extracted...

3/3,AB,KWIC/6
DIALOG(R)File 349:PCT FULLTEXT
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00270660
TREATMENT OF ANDROGEN-ASSOCIATED BALDNESS USING ANTISENSE OLIGOMERS TRAITEMENT DE LA CALVITIE ASSOCIEE AUX ANDROGENES AU MOYEN D'OLIGOMERES ANTISENS
Patent Applicant/Assignee:
GENTA INCORPORATED,
Inventor(s):
HARPER Mary Ellen,
WOOLF Tod Mitchell,
ARNOLD Lyle John Jr,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9418835 A1 19940901
Application: WO 94US1748 19940218 (PCT/WO US9401748) Priority Application: US 9319543 19930219
Designated States: AU CA JP KR NZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 12199

English Abstract
Methods of treating androgen-associated hair loss, in particular decreasing the progression of male pattern baldness using nucleoside Oligomers and Oligomers useful in the described methods are provided.
French Abstract
Procedes destines au traitement de l'alopecie associee aux androgenes, permettant en particulier de ralentir la progression de l'alopecie regionale masculine, et consistant a utiliser des oligomeres de nucleosides. Des oligomeres aptes a etre utilises selon les procedes susmentionnes sont egalement decrits.

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... invention include (a)
an antisense Oligomer having a sequence complementary to r@%% P@ @
ST I T U "I" EE7 (R U'11'.

a sequence of *RNA* transcribed from a target gene present in the cells; (b) an antisense Oligomer having a nucleo

side sequence complementary to a single stranded DNA target sequence; (c) an antisense Oligomer having a 5 nucleoside sequence complementary to a single *RNA* or DNA strand contained within. a duplex (d) a Third Strand

Oligomer having a sequence complementary to a selected double stranded nucleic acid sequence of...

...an Oligomer which decreases the rate of hair loss wherein said Oligomer is selected from an antisense Oligomer having a sequence complementar to that of *RNA* ly

transcribed from a gene for androgen receptor or an antisense Oligomer having a sequence complementary to a

sequence of *RNA* transcribed from a gene for 5-alphareductase.

According to a preferred aspect the Oligomer is a neutral Oligomer. Neutral Oligomers such as methylphosphonate Oligomers are...the

naturally-occurring bases, including the pyrimidine donor/acceptor bases such as pseudoisocytosine and pseudouracil and other modified bases (such as 8 substituted purines) . In *RNA*, the 5-carbon sugar is ribose; in DNA, it is 21 -deoxyribose. The term nucleoside also includes other analogs of such subunits, including

those which...of hydrogen bonding to a segment of a single CTITIF

OU130 I U r E SHEET (RULE 2-G)

stranded target nucleic acid, such as *RNA* or DNA, and, thus, together with the single stranded target nucleic

acid, are capable of forming a triple helix structure therewith.

The term "Third Strand Oligomer" refers to Oligomers which are capable of hybridizing to a segment of a double stranded nucleic acid, such as a DNA duplex, an *RNA* duplex or a DNA-*RNA* duplex, and forming a triple helix structure therewith.

The term "complementary, 11 when referring to a Triplex

Oligomer Pair (or first and second Oligomers) or...reference.

SUBZ-"3TfTUTE SHEET (RULE 26)

Also preferred are Oligomers which are nucleoside/ non-nucleoside polymers. Suitable Oligomers also include chimeric oligonucleotides which are composite *RNA*, DNA analogues (Inoue et al. , FEBS Lett. 2115:327 (1987)

5 other suitable Oligomers include Oligomers having chimeric backbones. Such chimeric backbone Oligomers include

Oligomers...

...including

nucleoside sequences which are capable of activating RNaseH and nucleoside sequences which do not activate RNaseH, 'and thus allow site directed cleavage of an *RNA* molecule. See U.S. Patent No. 5,149,797 which is incorporated herein by reference. Chimeric backbone Oligomers also include Oligomers having a mixture of...

...target polynucleotide

sequences.

According to one aspect of the present invention, these antisense Oligomers have a sequence which is complementary to a portion of the *RNA* transcribed from the selected target gene. Although the exact molecular

mechanism of inhibition has not been conclusively determined, it has been suggested to result from formation of duplexes between the antisense Oligomer and the *RNA* transcribed from the target gene. The duplexes so formed may inhibit translation, processing or transport of an

mRNA sequence or may lead to digestion by...

...acid sequence.

Triple strand formation can occur in one of several ways. A single stranded Oligomer may form a triple strand with duplex DNA or *RNA*; two separate or connected Oligomers may form a triple strand with single stranded DNA or *RNA*; two separate or connected Oligomers may bind to one of the

duplex DNA or *RNA* strands and displace the other such that it is not involved in triple strand formation. Further

descriptions of the use of Oligomers (including Third Strand...Oligomers and neutral Oligomers

comprising morpholino or phosphoramidate linkages.

Especially preferred are neutral methylphosphonate Oligomers. In view of their demonstrated ability to penetrate skin, including *tape* *stripped* skin, (which has had the stratum corneum removed and which has been

reported as a model for mucous membrane), particularly preferred are neutral methylphosphonate Oligomers...administered may, be either an antisense Oligomer,

a Third Strand Oligomer, or a Triplex Oligomer Pair. The antisense Oligomer is complementary to a sequence of *RNA*.

transcribed from a target gene, to a single-stranded DNA target sequence, or to a single *RNA* or DNA strand

contained within a duplex. The Third Strand Oligomer has 35 a base sequence selected so that it is capable of hydrogen bonding...procedures with the exception

that the coupling times were extended to 12 minutes to allow adequate time for the more sterically hindered 21 tert-butyldimethylsilyl *RNA* monomers to react. The

syntheses were begun on control-pore glass bound 21 tert-butyldimethylsilyl ribonucleosides purchased from Pennisula Laboratories. All other oligonucleotide synthesis reagents...by kinasing and analyzed by PAGE.

Example 2
Thermal denaturation profiles
The stabilities of triple stranded complexes formed between two MP oligomers and a complementary *RNA* oligomer were determined by thermal denaturation analysis. Solu
tions were prepared for analysis as follows: 2.4 gM MP 35 oligomer, 1.2 gM *RNA* oligomer (2:1 mole ratio MP:*RNA*) in 10 mM potassium phosphate, 0.1 M sodium chloride, 0.030i SUBSTITUTE SHEET (RULE 26) potassium sarkosylate, 0.1 mM EDTA, pH 7.2...

...melting temperatures (Tm) at which half of each complex had dissociated to single strands was 45.80C and 42.30C (2:1 mole ratio MP:*RNA*) for Oligomer 1 and Oligomer 2, respectively (see Table V. The entire melting curve
for MP Oligomer 2 and its target at 2:1 and
...hybridized.
Figure 1 depicts a thermodenaturation profiles for double-stranded and triple-stranded complexes formed between Oligomer 2 and a target sequence.

Table I
(MP:*RNA*
mole
Oligomer Tm (OC) ratio)
[41 Oligomer 1 (Androgen Rec. #1 44.10C (1:1)
target: 5f gag-aga-gag-tgg-ggg-aa) 45.80C...dermis was about 10:30 (Note: Since there was considerably more viable
tissue than stratum corneum, the majority of oligomer retained was in the dermis) . *Tape* *stripping* (to remove SUBSTITUTE SHEET (RULE 26)
stratum corneum) of skin did not lead to a larger amount of 6-mer being retained in dermis as...footnotes, all the donor vehicles were saturated with oligomer
'OA = oleic acid
cEtAc = ethylacetate
dThese skins were free of stratum corneum, which was removed by *tape* *stripping*.

e14-mer concentration in the vehicle was 1.0 mg/mL (below saturation)
f14-mer concentration in the vehicle was 0.5 mg/mL (below...Data PG@/DMS (95:5) 0.57(0.50) No Data No Data
EtOH/DMS (95:5) 34.0(4.8) No Data No Data
(*Tape* *Stripped*)
The second value for the 6Mer came from a time study using a different skin donor, otherwise the data for the first three enhancers came...

...DNS (95:5) 6 (2) 150 (120)
EtOH (100:0) 5 (4) 160 (180)
GI
M 6 Her EtOH/DMS (TS) (95:5) 73 (14) *Tape* *Stripped* PG (100to) 6 (6) 47 (10)
PG/DMS (95:5) 6 (3) 138 (4)
6 Mar EtOH/H20/DMS (80:15s5) Mean (A.D.)o...Mar time study had one cell for the 30 minute point and 2 cells for the other ti "A'aDo is the average deviation.

bTs=*tape* *stripped* skin (stratum corneum removed,) Claim
... 5-alpha-reductase.

2 A method according to claim 1 wherein said Oligomer or Oligomers specifically recognize a target sequence which comprises (a) single stranded *RNA* or DNA,, (b) double stranded DNA or *RNA*, (c) a DNA/*RNA* duplex or (d) a single *RNA* or DNA strand contained within a duplex and wherein said Oligomer interferes with transcription or translation of the target sequence.

3 A method according to...

...a decrease in rate of hair loss whereby said Oligomer or Oligomers specif ically recognize a nucleic acid target sequence selected from (a) single stranded *RNA* or DNA (b) double stranded DNA or *RNA*, (c) a DNA/*RNA* duplex, or (d) a single *RNA* or DNA strand contained within a duplex whereby the Oligomer or Oligomers selectively interfere
with transcription or translation of the nucleic acid target sequence and...

...upstream from the transcription
start site of the gene.

9 a method according to claim 8 wherein when said target sequence is (a) single stranded *RNA* or DNA or (b) a single *RNA* or DNA strand contained within a duplex, the Oligomer or Oligomers are an antisense Oligomer or a
Triplex Oligomer Pair.

10 A method according to claim 8 wherein when said target sequence is (a) double stranded DNA or *RNA* or (b) 30 a DNA/*RNA* duplex, the Oligomer is a Third Strand ...a decrease in rate of hair loss wherein said Oligomer is
selected from (a) an antisense Oligomer having a sequence complementary to a sequence of *RNA* transcribed from a target gene present in the cells; (b) an antisense
Oligomer having a nucleoside sequence complementary to a single stranded DNA target sequence; (c) an antisense Oligomer having a nucleoside sequence complementary to a single *RNA* or DNA strand contained within a duplex;

(d) a Third Strand Oligomer having a sequence complementary to a selected double stranded nucleic acid sequence of...of RN A transcribed from a gene for the androgen receptor or (b) 5 an antisense oligomer having a sequence complementary to a sequence of *RNA* transcribed from a gene for 5-areductase.
26 A method of claim 25 wherein said Oligomer is a substantially neutral Oligomer.
27 A method according...

...complementary to a 51-untranslated region, a translation initiation region a 31-untranslated region, a splice donor site, or a splice acceptor site of said *RNA*.
28 A method according to claim 27 wherein said 15 Oligomer comprises from about 12 to about 25 nucleosides.
29 A method of decreasing levels...

...5-alpha reductase.

30 A method according to claim 29 wherein said Oligomer or Oligomers specifically recognize a target sequence which comprises (a) single stranded *RNA* or DNA, 30 (b) double stranded DNA or *RNA*, (c) a DNA/*RNA* duplex or (d) a single *RNA* or DNA strand contained within a duplex SU 0TijU*I1:SnEt7:(RUL*4hII
and wherein said Oligomer interferes with transcription or translation of the target...
...diminish rate of
androgen-associated hair loss wherein said Oligomer is selected from (a) an antisense Oligomer having a sequence complementary to a sequence of *RNA* transcribed from a target gene present in the cells; (b) an antisense
Oligomer having a nucleoside sequence complementary to a single stranded DNA target sequence; (c) an antisense Oligomer having a nucleoside sequence complementary to a single *RNA* or DNA strand contained within a duplex; (d)

3/3,AB,KWIC/7
DIALOG(R)File 349:PCT FULLTEXT

00249839
TREATMENT OF CELLULAR HYPERPROLIFERATION BY INHIBITION OF INTERLEUKIN-1 TRAITEMENT DE L'HYPERPROLIFERATION CELLULAIRE PAR INHIBITION DE L'INTERLEUKINE-1
Patent Applicant/Assignee:
GENTA INCORPORATED,
UNIVERSITY OF MICHIGAN,
Inventor(s):
COOPER Kevin D,
HAMMERBERG Craig,
MAXWELL Kameron W,
TSENG Ben Y,

Patent and Priority Information (Country, Number, Date):
Patent: WO 9324134 A1 19931209
Application: WO 93US4917 19930521 (PCT/WO US9304917) Priority Application: US 92887734 19920522
Designated States: AU CA JP KR NZ AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 9956
English Abstract
Methods of treating conditions characterized by cellular hyperproliferation using Interleukin-1 inhibitory compounds and compounds useful in the described methods are provided.

French Abstract
L'invention concerne des methodes de traitement d'etats pathologiques caracterises par une hyperproliferation cellulaire, consistant a utiliser des composes inhibiteurs de l'interleukine-1. L'invention concerne egalement les composes utilises dans les methodes de traitement decrites.

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... an antisense Oligomer, a Third Strand Oligomer or a Triplex.oligomer. Such an antisense Oligo 5 mer has a sequence complementary to a sequence of *RNA* transcribed from a target gene present in the cells. A
Triple Strand Oligomer has a sequence complementary to a selected double stranded nucleic acid sequence...

...A, G, C, T and U as their bases, but also analogs and modified forms of the bases (such as 8-sub stituted purines). In *RNA*, the 5 carbon sugar is ribose; in DNA, it is a 21-deoxyribose. The term also includes
analogs of such subunits, including modified sugars such...second Oligomers which are complementary to and which are
caDable of hydrogen bonding to a segment of a single stranded target nucleic acid, such as *RNA* or DNA, and, thus, together. with the single stranded target nucleic
acid are capable of forming a triple helix structure therewith.

The term "Third Strand Oligomer" refers to Oligomers which are capable of hybridizing to a segment of a double stranded nucleic acid, such as a DNA duplex, an *RNA* duplex or a DNA-*RNA* duplex, and forming a triple helix structure therewith.

The term "complementary, 11 when referring to a

Triplex
Oligomer 'Pair (or f irst and second Oligomers...
...to about 40
nucleosidyl units. Also preferred are Oligomers which are nucleoside/non-nucleoside polymers. Suitable Oligomers
also include chimeric oligonucleotides which are comr)osite *RNA*, DNA analogues (Inoue et al., FEBS Lett, 2115:327 (1987)). Oligomers having a neutral backbone, for example, methylphosphonate Oligomers with cleaving or cross
linking moieties of the present invention,
these antisense Oligomers have a sequence which is com
plementary to a portion of the *RNA* transcribed from the selected target gene. Although the exact molecular mech
anism of inhibition has not been conclusively determined, it has been suggested to result from formation of duplexes between the antisense Oligomer and the *RNA* transcribed from the target gene. The duplexes so formed may inhibit translation, processing or transport of an mRNA sequence or may lead to digestion by...Oligomers and neutral Oligomers comprising morpholino or phosphoramidate linkages.

Especially preferred are neutral methylphosphonate Oligomers. In view of their demonstrated ability to penetrate skin, including *tape* *stripped* skin, (which has had the stratum corneum removed and which has been
reported as a model for mucous membrane), particularly preferred are neutral methylphosphonate Oligomers...of an Oligomer, either an antisense
Oligomer, a Third Stand Oligomer or a Triplex Oligomer Pair. The antisense Oligomer is complementary to a sequence of *RNA* transcribed from a target gene. The Third Strand Oligomer has a base sequence selected so that it is 35 capable of hydrogen bonding with a...

Claim
... epithelial cells to a hyper
proliferation inhibiting amount of an Oligomer selected from (a) an antisense Oligomer having a sequence complementary to a sequence of *RNA* tran
scribed from a target gene present in the cells;
(b) a Third Strand Oligomer having a sequence com
plementary to a selected double stranded...

...cells to a hyper
proliferation-inhibiting amount of an Oligomer selected from (a) an antisense Oligomer having a sequence complementary to a se ence of *RNA*
qu
transcribed from a target gene present in the cells; (b).a Third Strand Oligomer having a sequence complementary to a selected double stranded nucleic...which comprises a
hyperproliferation inhibiting amount of an Oligomer sel
ected from (a) an antisense Oligomer having a sequence
30 complementary to a sequence of *RNA* transcribed from a target gene present in the cells; (b) a Third Strand
SUBSTITUTE SHEET
Oligomer having a sequence complementary to a selected double stranded...

3/3,AB,KWIC/8
DIALOG(R)File 349:PCT FULLTEXT
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00224111
COMPOSITIONS AND DELIVERY SYSTEMS FOR TRANSDERMAL ADMINISTRATION OF NEUTRAL OLIGOMERS
COMPOSITIONS ET SYSTEMES D'APPORT DESTINES A L'ADMINISTRATION PAR VOIE TRANSDERMIQUE D'OLIGOMERES NEUTRES
Patent Applicant/Assignee:
GENTA INCORPORATED,
Inventor(s):
ARNOLD Lyle J Jr,
MAXWELL Kameron,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9221353 A1 19921210
Application: WO 92US4370 19920522 (PCT/WO US9204370) Priority Application: US 91879 19910531
Designated States: AT AU BE CA CH DE DK ES FI FR GB GR IT JP KR LU MC NL NO RU SE
Publication Language: English
Fulltext Word Count: 10044

English Abstract
Neutral Oligomers may be delivered transdermally and across mucous membranes for therapeutic purposes, such as to block expression of a specific target nucleic acid sequence. These neutral Oligomers are useful in treating a variety of pathological conditions, including those of the skin and mucous membranes, as well as conditions effecting other tissues.

French Abstract
Des oligomeres neutres peuvent etre achemines par voie transdermique et a travers des muqueuses a des fins therapeutiques, de maniere a bloquer l'expression d'une sequence d'acides nucleiques cible specifique. Ces oligomeres neutres sont utiles dans le traitement de divers etats pathologiques, y compris ceux de la peau et des muqueuses, ainsi que des etats affectant d'autres tissus.

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... interchangeably therewith, and ref ers to a subunit
35 of a nucleic acid which comprises a 5 carbon sugar and a nitrogen-containing base. In *RNA* the 5 carbon sugar is SUBSTITUTE SHEET
ribose; in DNA, it is a 21-deoxyriboseo The term also includes analogs of such subunitso
A "non...Oligomers comprising triester or phosphoramidate internucleosidyl link
ages. Especially preferred are neutral methylphosphonate Oligomers. In view of their demonstrated ability to pene trate skin, including *tape* *stripped* skin (which has had the stratum corneum removed and which has been reported as a model for mucous membrane), particularly preferred are neutral methylphosphonate Oligomers...to which the
stratum corneum limits the absorption of methylphosphonate Oligomers, an experiment was performed wherein the stratum corneum. was removed from the skin by *tape* *stripping*.
Also, *tape* *stripped* skin has been reported to have similar permeability characteristics to mucous membrane andF thus., has been proposed as a model system for mucous membrane.
Penetration...

...and ethanol (EtOH) a The amount of the neutral methylphosphonate 14-mer penetrating skin was increased drama
tically when the stratum corneum was removed by *tape* *stripping*. Removal of the stratum corneum by tape strip ping resulted in absorption of the entire dose of 14@mer SUBSTITUTE SHEET
absorbed through the stripped...dermis was about
10-30 (Note: Since there was considerably more viable
tissue than stratum corneum,, the majority of oligomer
retained was in the dermis). *Tape* *stripping* (to remove stratum corneum) of skin did not lead to a larger amount of 6-mer being retained in dermis as compared to retention in...footnotes,, all the donor
vehicles were saturated with oligomer
bOA = oleic acid
cEtAc = ethylacetate
dThese, skins were free of stratum corneum,, which was
removed by *tape* *stripping*.

SUBSTITUTE SHEET
e14-mer concentration in the vehicle was 1.0 mg/mL (below saturation)
f14-mer concentration in the vehicle was 0.5 mg...Data No Data PG/DMS (95:5) 0.57(0.50) No Data No Data
EtOH/DMS (95:5) 34eO(4e8) No Data No Data
Tape
Stripped
SUBSTITUTE SHEET
The second value for the 6Mer came from a time study using a different skin donor, otherwise the data for the first three...

...DMS (95:5) 6 (2) 150 (120)
EtOH 2a
(100:0) 5 (4) 160 (180) 6j
6
Her EtOH/DMS (TS (95:5) 73 (14) *Tape* *Stripped* Tal co PG (100:0) 6 (6) 47 (10)
X PG/DMS (95:5) 6 (3)
M 138 (4) 2d
Her EtOH/H O/DMS point and 2 cells for the other time points.
OA.D. is the average deviation.

bTS=*tape* *stripped* skin (stratum corneum removed.)
Claim
... a growth factor, a
cell adhesion molecule or a receptor thereof. 7e A method according to claim 1 wherein said tar
get sequence comprises an *RNA* region which codes for an 30 initiation codon region, a polyadenylation region, a mRNA cap site, or a splice junction.
SUBSTITUTE SHEET
so A method...selectively binding to or interacting with a specific nucleic acid target sequence.

20 An Oligomer according to claim 19 wherein said
target sequence comprises an *RNA* region which codes for an initiator codon·region, a polyadenylation region, a mRNA cap site or a splice junction or a DNA region which
results...

3/3,AB,KWIC/9
DIALOG(R)File 349:PCT FULLTEXT
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00197852
COMPOSITIONS COMPRISING CYTOTOXIC AGENT AND PERMEATION ENHANCERS COMPOSITIONS COMPRENANT UN AGENT CYTOTOXIQUE ET DES AGENTS AUGMENTANT LA PERMEATION
Patent Applicant/Assignee:
ALZA CORPORATION,
Inventor(s):
CORMIER Michel J N,
TASKOVICH Lina T,
YUM Su Il,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9115210 A1 19911017
Application: WO 91US1987 19910325 (PCT/WO US9101987) Priority Application: US 90488 19900330
Designated States: AT AU BE CH DE DK ES FI FR GB GR IT JP KR LU NL NO SE Publication Language: English
Fulltext Word Count: 4744

English Abstract
The present invention is directed to a composition of matter for the percutaneous administration of a

cytotoxic agent, and particularly to the percutaneous administration of 5-fluorouracil. The composition comprises, in combination, the cytotoxic agent to be administered and a permeation-enhancing mixture that includes a lower alkanol, propylene glycol or a mixture of polyethylene glycols, a third permeation enhancer and, optionally, a covehicle. The invention is also directed to a method for treating malignant and non-malignant skin disorders and comprises applying to an area of skin affected by a skin disorder, a therapeutically effective amount of the composition of this invention.
French Abstract
La presente invention concerne une composition a base d'une substance utilisee pour administrer de maniere percutanee un agent cytotoxique, et en particulier pour administrer de maniere percutanee du fluoro-5-uracile. La composition est constituee de l'agent cytotoxique devant etre administre en combinaison avec un melange rehausseur de permeation qui comprend une alcanol inferieur, un propyleneglycol ou un melange de polyethylene glycols; un troisieme rehausseur de permeation et, facultativement, un covehicule. L'invention concerne aussi une methode de traitement d'affections de la peau malignes ou non, et consiste a appliquer a une region de la peau presentant une affection une quantite therapeutiquement suffisante de la composition de cette invention.
Fulltext Availability:
Detailed Description

Detailed Description
... activity. Thymidylate synthetase catalyzes the methylation of deoxyuridylic acid to thymidylic acid, a DNA precursor. It also inhibits, to a lesser extent, the formation of *RNA*. The effects of DNA and *RNA* deprivation are most marked on those
cells which grow more rapidly and which take up fluorouracil at a more rapid pace.

Fluorouracil is used topically...were used (one site per animal).
The stripping was performed on the entire site and the sectioning on three biopsies-of 0,28 CM2 , After *tape* *stripping*, the guinea pig
skin presented only a few remaining cornified layers at the surface of the epidermis. The epidermis was 30 to 40 jim thick... ? b 155
09dec02 12:55:14 User217743 Session D585.4
$5.11 1.075 DialUnits File349
$7.35 1 Type(s) in Format 9
$49.05 9 Type(s) in Format 5 (UDF)
$56.40 10 Types
$61.51 Estimated cost File349
$0.43 TELNET
$61.94 Estimated cost this search
$68.65 Estimated total session cost 4.738 DialUnits
File 155:MEDLINE(R) 1966-2002/Nov W3
*File 155: For updating information please see Help News155. Alert feature enhanced with customized scheduling. See HELP ALERT.
Set Items Description
--- ----- -----------
? s tape()stripp? and rna
8109 TAPE
5345 STRIPP?
389 TAPE(W)STRIPP?
347480 RNA
S1 21 TAPE()STRIPP? AND RNA
? s s1 and py>1998
21 S1
1886970 PY>1998
S2 3 S1 AND PY>1998
? s s1 not s2
21 S1
3 S2
S3 18 S1 NOT S2
? t s3/3,ab,kwic/all

3/3,AB,KWIC/1
DIALOG(R)File 155:MEDLINE(R)

10221333 99198828 PMID: 10100729
Epidermal cell kinetics by combining in situ hybridization and immunohistochemistry.
Castelijns F A; Ezendam J; Latijnhouwers M A; Van Vlijmen-Willems I M; Zeeuwin P L; Gerritsen M J; Van de Kerkhof P C; Van Erp P E Department of Dermatology, University Hospital Nijmegen, The Netherlands.
Histochemical journal (ENGLAND) Dec 1998, 30 (12) p869-77, ISSN 0018-2214 Journal Code: 0163161
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Double labelling can serve as a useful tool for providing information about cell kinetics in normal and hyperproliferative tissues in general, and skin in particular. We have developed a double-labelling method that combines immunohistochemistry using the monoclonal antibody MIB1 and non-isotopic in situ hybridization using either a digoxigenin-labelled *RNA* probe specific for histone 3 mRNA sequences or a Fluorescein-labelled oligonucleotide probe specific for histone 2b, 3, 4 mRNA sequences. Double labelling was performed on normal, *tape*- *stripped* normal skin and psoriatic skin. The three proliferation markers were also examined by single labelling. The ratio of cells in the S-phase (Ns) and the growth fraction (Ncy) was determined. In normal skin, psoriatic skin and *tape*-*stripped* normal skin after 24 h and after 48 h, we calculated that 15%, 16%, 3% and 12% of growth fraction consisted of cells in the S-phase respectively. The S-phase lasts approximately 10 h, so the cell cycle time in normal and psoriatic skin is approximately 62.5 h. At present, the MIB1/H3 digoxigenin or MIB1/H2b-H3-H4 Fluorescein

double-labelling technique cannot be used routinely. Therefore, in order to understand the cell kinetic processes better, experiments are recommended to optimize these methods. From a practical point of view and for reasons of specificity and sensitivity, we prefer the Fluorescein-labelled oligonucleotide probe method.

... have developed a double-labelling method that combines immunohistochemistry using the monoclonal antibody MIB1 and non-isotopic in situ hybridization using either a digoxigenin-labelled *RNA* probe specific for histone 3 mRNA sequences or a Fluorescein-labelled oligonucleotide probe specific for histone 2b, 3, 4 mRNA sequences. Double labelling was performed on normal, *tape*-*stripped* normal skin and psoriatic skin. The three proliferation markers were also examined by single labelling. The ratio of cells in the S-phase (Ns) and the growth fraction (Ncy) was determined. In normal skin, psoriatic skin and *tape*-*stripped* normal skin after 24 h and after 48 h, we calculated that 15%, 16%, 3% and 12% of growth fraction consisted of cells in the...

3/3,AB,KWIC/2
DIALOG(R)File 155:MEDLINE(R)

10035036 99019473 PMID: 9804328
Expression and regulation of mRNA for putative fatty acid transport related proteins and fatty acyl CoA synthase in murine epidermis and cultured human keratinocytes.
Harris I R; Farrell A M; Memon R A; Grunfeld C; Elias P M; Feingold K R Department of Veterans Affairs Medical Center and Department of Dermatology, University of California, San Francisco 94121, USA.
Journal of investigative dermatology (UNITED STATES) Nov 1998, 111 (5) p722-6, ISSN 0022-202X
Journal Code: 0426720
Contract/Grant No.: AR19098; AR; NIAMS; AR39639; AR; NIAMS Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The epidermis has a requirement for fatty acids in order to synthesize cellular membranes and the extracellular lipid lamellar membranes in the stratum corneum. Despite high endogenous production of fatty acids the transport of exogenous essential fatty acids into the epidermis is an absolute requirement. Fatty acid uptake by keratinocytes has been shown to be mediated by a transport system. In this study we determined in murine epidermis and human cultured keratinocytes the expression of three putative fatty acid transport related proteins and fatty acyl CoA synthase, an enzyme that facilitates the uptake of fatty acids by promoting their metabolism. In cultured human keratinocytes, mRNA for fatty acid transport protein (FATP), plasma membrane fatty acid binding protein (FABP-pm), and fatty acyl CoA synthase (FACS) were detectable. Differentiation, induced by high calcium, did not affect FATP mRNA levels, but resulted in an approximately 50% increase in FACS mRNA, while decreasing FABP-pm mRNA by 50%. Fatty acid translocase (FAT) mRNA was not detected in cultured human keratinocytes. In murine epidermis, FATP, FABP-pm, FACS, and FAT mRNA were all present. Barrier disruption by either *tape* *stripping* or acetone treatment increased FAT mRNA levels by approximately 2-fold without affecting FATP, FABP-pm, or FACS. Occlusion with an impermeable membrane immediately after barrier disruption completely blocked the increase in FAT mRNA levels, indicating that this increase is related to barrier disruption rather than a nonspecific injury effect. In summary, this study demonstrates that several putative fatty acid transport related proteins as well as fatty acyl CoA synthase are expressed in keratinocytes and epidermis, and that the expression of these proteins may be regulated by differentiation and/ or barrier disruption.

... mRNA was not detected in cultured human keratinocytes. In murine epidermis, FATP, FABP-pm, FACS, and FAT mRNA were all present. Barrier disruption by either *tape* *stripping* or acetone treatment increased FAT mRNA levels by approximately 2-fold without affecting FATP, FABP-pm, or FACS. Occlusion with an impermeable membrane immediately after ...
Descriptors: Carrier Proteins--genetics--GE; *Coenzyme A Ligases --genetics--GE; *Keratinocytes--metabolism--ME; *Membrane Proteins --genetics--GE; **RNA*, Messenger--metabolism--ME; *Skin--metabolism --ME
Chemical Name: Carrier Proteins; FAT protein, adipocyte; Membrane Glycoproteins; Membrane Proteins; *RNA*, Messenger; fatty acid-transport protein; Coenzyme A Ligases; long-chain-fatty-acid-CoA ligase

3/3,AB,KWIC/3
DIALOG(R)File 155:MEDLINE(R)

09766489 98202651 PMID: 9541570
Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response.
Kondo H; Ichikawa Y; Imokawa G
Biological Science Laboratory, Kao Corporation, Tochigi, Japan. European journal of immunology (GERMANY) Mar 1998, 28 (3) p769-79, ISSN 0014-2980 Journal Code: 1273201
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed

We investigated whether percutaneous sensitization with different allergens through barrier-disrupted skin regulates the balance of Th1/Th2 cytokine expression. When mice were sensitized with the typical hapten picryl chloride (PiCl) by a single topical application to intact skin, there was an up-regulation in the lymph nodes (LN) of mRNA expression for the Th1 cytokines IL-2 or IFN-gamma, and for the Th2 cytokine IL-4. In contrast, sensitization with PiCl after barrier disruption of the skin down-regulated the expression of mRNA for IFN-gamma in a *tape*- *stripping* number-dependent manner without changing the expression of mRNA for IL-4. When mice were sensitized with house dust mite antigens (MA) by a single topical application to barrier-disrupted abdominal skin, there was a *tape*-*stripping* number-dependent up-regulation in the LN of mRNA expression for IL-4 but not for IL-2 or IFN-gamma. In the LN, mRNA for the IL-4-inducible immunoglobulins IgE and IgG1, but not for the IFN-gamma-inducible IgG2a, were up-regulated after sensitization with MA, while all three immunoglobulin mRNA were augmented after PiCl sensitization through intact skin. Antigenic elicitation by a topical application of PiCl in aural skin of mice sensitized through intact skin consistently increased the expression of mRNA for all three cytokines in the challenged skin, whereas elicitation in mice sensitized through barrier-disrupted skin decreased the expression of mRNA for IL-2 and IFN-gamma, but not for IL-4. Antigenic elicitation by subcutaneous injection of MA in aural skin consistently increased the expression of mRNA for IL-4, but not for IL-2 or IFN-gamma in the challenged skin. Infiltration of eosinophils in the dermis was more prominent following elicitation with MA in mice sensitized through barrier disruption than with PiCl in mice sensitized through intact skin. These findings suggest that the percutaneous entry of environmental allergens through barrier-disrupted skin is strongly associated with the induction of Th2-dominant immunological responses, as is seen in atopic dermatitis.

... cytokine IL-4. In contrast, sensitization with PiCl after barrier disruption of the skin down-regulated the expression of mRNA for IFN-gamma in a *tape*-*stripping* number-dependent manner without changing the expression of mRNA for IL-4. When mice were sensitized with house dust mite antigens (MA) by a single topical application to barrier-disrupted abdominal skin, there was a *tape*-*stripping* number-dependent up-regulation in the LN of mRNA expression for IL-4 but not for IL-2 or IFN-gamma. In the LN, mRNA...

...; Interleukin-4--genetics--GE; Interleukin-6--genetics--GE; Lymph Nodes--immunology--IM; Mice; Mice, Inbred BALB C; Mites--immunology--IM; Permeability; Picryl Chloride--immunology--IM; *RNA*, Messenger --genetics--GE; Time Factors

Chemical Name: Allergens; Immunoglobulins; Interleukin-2; Interleukin-6; *RNA*, Messenger; Interleukin-4; Interferon Type II; Picryl Chloride

3/3,AB,KWIC/4
DIALOG(R)File 155:MEDLINE(R)

09644776 98068656 PMID: 9406821

Permeability barrier disruption coordinately regulates mRNA levels for key enzymes of cholesterol, fatty acid, and ceramide synthesis in the epidermis.

Harris I R; Farrell A M; Grunfeld C; Holleran W M; Elias P M; Feingold K R

Department of Veterans Affairs Medical Center and Department of Dermatology, University of California, San Francisco 94121, USA. Journal of investigative dermatology (UNITED STATES) Dec 1997, 109 (6) p783-7, ISSN 0022-202X Journal Code: 0426720

Contract/Grant No.: AR 39639; AR; NIAMS; AR19098; AR; NIAMS Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The extracellular lipids of the stratum corneum, which are comprised mainly of cholesterol, fatty acids, and ceramides, are essential for epidermal permeability barrier function. Moreover, disruption of the permeability barrier results in an increased cholesterol, fatty acid, and ceramide synthesis in the underlying epidermis. This increase in lipid synthesis has been shown previously to be due to increased activities of HMG-CoA reductase, acetyl-CoA carboxylase, fatty acid synthase and serine palmitoyl transferase, key enzymes of cholesterol, fatty acid, and ceramide synthesis, respectively. In the present study, we determined whether the mRNA levels for the key enzymes required for synthesis of these three classes of lipids increase coordinately during barrier recovery. By northern blotting, the steady-state mRNA levels for HMG-CoA reductase, HMG-CoA synthase, farnesyl pyrophosphate synthase, and squalene synthase, key enzymes for cholesterol synthesis, all increased significantly after barrier disruption by either acetone or *tape* *stripping* . Additionally, the steady-state mRNA levels of acetyl-CoA carboxylase and fatty acid synthase, required for fatty acid synthesis, as well as serine palmitoyl transferase, the rate-limiting enzyme of de novo ceramide synthesis, also increased. Furthermore, artificial restoration of the permeability barrier by occlusion after barrier disruption prevented the increase in mRNA levels for all of these enzymes, except farnesyl pyrophosphate synthase, indicating a specific link of the increase in mRNA levels to barrier requirements. The parallel increase in epidermal mRNA levels for the enzymes required for cholesterol, fatty acid, and ceramide synthesis may be

due to one or more transcription factors that regulate lipid requirements for permeability barrier function in keratinocytes.

... reductase, HMG-CoA synthase, farnesyl pyrophosphate synthase, and squalene synthase, key enzymes for cholesterol synthesis, all increased significantly after barrier disruption by either acetone or *tape* *stripping* . Additionally, the steady-state mRNA levels of acetyl-CoA carboxylase and fatty acid synthase, required for fatty acid synthesis, as well as serine palmitoyl transferase...

Descriptors: Ceramides--biosynthesis--BI; *Cholesterol--biosynthesis--BI; *Epidermis--metabolism--ME; *Fatty Acids--biosynthesis--BI; **RNA* , Messenger--analysis--AN

Chemical Name: Ceramides; Fatty Acids; *RNA*, Messenger; Cholesterol; Hydroxymethylglutaryl CoA Reductases; Acyltransferases; serine palmitoyltransferase; Fatty Acid Synthetase Complex

3/3,AB,KWIC/5
DIALOG(R)File 155:MEDLINE(R)

09452061 97353651 PMID: 9209892

Barrier disruption increases gene expression of cytokines and the 55 kD TNF receptor in murine skin.

Wood L C; Stalder A K; Liou A; Campbell I L; Grunfeld C; Elias P M; Feingold K R

Dermatology and Medical Services, Department of Veterans Affairs Medical Center, San Francisco, CA 94121, USA.

Experimental dermatology (DENMARK) Apr 1997, 6 (2) p98-104, ISSN 0906-6705 Journal Code: 9301549

Contract/Grant No.: AR 19098; AR; NIAMS; AR 39448; AR; NIAMS; AR 39639; AR; NIAMS; +

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The signalling mechanisms that regulate epidermal permeability barrier homeostasis are not known. Previous Northern blot analysis showed that both acute and chronic barrier disruption increase mRNA levels of several cytokines in murine epidermis. To further characterize the epidermal response to barrier abrogation, we used more sensitive, multi-probe RNase protection assays to measure the mRNA levels of additional cytokines, as well as cytokine receptors in acute and chronic models of barrier disruption. Normal mouse epidermis expressed interleukin (IL)-1 alpha, interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha) and IL-6 mRNAs. Following *tape*-*stripping*, only the mRNA levels for TNF-alpha, IL-1 alpha, IL-1 beta and IL-6 increased at 2.5 and 7 h, and returned toward normal levels by 18 h. No mRNAs encoding TNF-beta, IL-2, IL-3, IL-4 or IL-5, were detected in the epidermis either under basal conditions or after *tape*-*stripping* . Similarly, in a chronic model, essential fatty acid deficiency, epidermal levels of TNF-alpha, IL-1 alpha, IL-1 beta and IL-6 mRNAs, but not IFN-gamma mRNA, were elevated over controls; and again, mRNAs for the remaining probed cytokines were not detected. In contrast, in the dermis, only IL-1 beta mRNA levels increased 2.5 h after *tape*-*stripping* , and remained elevated at 18 h. mRNAs encoding the IL-1 (p60), IFN-gamma and IL-6 receptors were present in epidermis, but their levels remained unchanged following either acute or chronic barrier disruption. In contrast, epidermal TNF (p55) receptor mRNA levels were increased by 87% ($P < 0.01$) at 2.5 h, returned to control levels at 7 h and were increased by 68% ($P < 0.03$) at 18 h after *tape* -*stripping* . The increase at 2 h was confirmed by Northern blot analysis and was not prevented by latex occlusion performed immediately after *tape*-*stripping* mRNAs for the IL-1 (p80) receptor and TNF (p75) receptor were not detected in epidermis. Low levels of TNF (p55) receptor mRNA were present in the dermis, and they remained unchanged after *tape*-*stripping* . The presence of specific receptor mRNAs in the epidermis and dermis suggests that these tissues are capable of responding in an autocrine and/or paracrine fashion to the cognate cytokines. These results suggest that epidermal cytokines produced after barrier disruption may initiate a cytokine cascade which could regulate cytokine and cytokine receptor production and/or inflammatory responses.

... barrier disruption. Normal mouse epidermis expressed interleukin (IL)-1 alpha, interferon-gamma (IFN-gamma), tumor necrosis factor-alpha (TNF-alpha) and IL-6 mRNAs. Following *tape*-*stripping*, only the mRNA levels for TNF-alpha, IL-1 alpha, IL-1 beta and IL-6 increased at 2.5 and 7 h, and returned...

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...; Epidermis--metabolism--ME; Fatty Acids, Essential--administration and dosage--AD; Fatty Acids, Essential--deficiency--DF; Latex; Mice; Mice, Inbred HRS; Mice, Mutant Strains; Occlusive Dressings; *RNA*, Messenger --biosynthesis--BI; *RNA*, Messenger--genetics--GE; Receptors, Interleukin--biosynthesis--BI; Receptors, Interleukin--genetics--GE; Receptors, Interleukin-1--biosynthesis--BI; Receptors, Interleukin-1 --genetics--GE; Receptors, Interleukin-6; Receptors...

Chemical Name: Antigens, CD; Cytokines; Dietary Fats; Fatty Acids, Essential; Latex; *RNA*, Messenger; Receptors, Interleukin; Receptors, Interleukin-1; Receptors, Interleukin-6; Receptors, Tumor Necrosis Factor

3/3,AB,KWIC/6
DIALOG(R)File 155:MEDLINE(R)

09246026 97134731 PMID: 8980291

Amphiregulin and nerve growth factor expression are regulated by barrier status in murine epidermis.

Liou A; Elias P M; Grunfeld C; Feingold K R; Wood L C

Clinical Pharmacy, University Paris XI, France.

Journal of investigative dermatology (UNITED STATES) Jan 1997, 108 (1) p73-7, ISSN 0022-202X Journal Code: 0426720

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Disruption of the murine permeability barrier by solvents or *tape* *stripping* stimulates a homeostatic repair response that includes increased epidermal DNA synthesis. To identify potential mediators of the increase in DNA synthesis, we have measured epidermal levels of mRNAs encoding various growth factors after acute barrier disruption. In this study, mRNAs for amphiregulin and nerve growth factor were each shown to increase over controls at 30 min, reach peak levels of 12- to 30-fold at 1-2 h, and return to control levels by 6 h after *tape* *stripping* . A similar time course for the increase of amphiregulin and nerve growth factor mRNAs was observed after an unrelated form of barrier disruption, i.e., acetone treatment. Furthermore, artificial restoration of the barrier by Latex occlusion, immediately following barrier disruption by acetone treatment, inhibited the increase in epidermal amphiregulin and nerve growth factor mRNA levels, indicating that barrier status regulates the production of these growth factors. In contrast, mRNA levels of transforming growth factor-beta1, an inhibitory growth factor, were unchanged at early times and decreased by 53% ($p < 0.02$) 6 h after *tape* *stripping* , whereas mRNA levels of transforming growth factor-alpha remained unchanged at all times after acute barrier disruption. These results suggest that barrier disruption stimulates the expression of amphiregulin and nerve growth factor. Together, these regulators of keratinocyte growth and differentiation may be responsible for the increased proliferative response that is associated with barrier disruption.

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Chemical Name: Glycoproteins; Growth Substances; Nerve Growth Factors; *RNA*, Messenger; Transforming Growth Factor alpha; Transforming Growth Factor beta; amphiregulin

3/3,AB,KWIC/7
DIALOG(R)File 155:MEDLINE(R)

09235897 97118506 PMID: 8959348

Modulation of the plasminogen activator cascade during enhanced epidermal proliferation in vivo.

Jensen P J; Lavker R M

Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia 19104-6142, USA.

Cell growth & differentiation : the molecular biology journal of the American Association for Cancer

Research (UNITED STATES) Dec 1996, 7 (12) p1793-804, ISSN 1044-9523 Journal Code: 9100024
Contract/Grant No.: RO1-AR-42998; AR; NIAMS; RO1-EY-06769; EY; NEI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Many lines of evidence support an involvement of urokinase plasminogen activator (uPA) and its type 1 inhibitor (PAI-1) in the migration of a variety of cells, including normal keratinocytes and carcinoma lines. In the present study, uPA expression was found to be a characteristic not just of migratory but also of proliferative keratinocytes. A variety of naturally occurring and experimentally induced epidermal hyperproliferative conditions were examined in mice, including fetal and neonatal epidermis, *tape*-*stripped* epidermis, and epidermis from which the hairs had been gently plucked. In all cases, epidermal hyperproliferation was accompanied by elevated levels of uPA mRNA (as measured by in situ hybridization) and activity (as measured by zymography). uPA mRNA was predominantly localized in the basal and immediately suprabasal cells, which constitute the proliferative population. To determine whether a PAI was concomitantly elevated, in situ hybridization for PAI-1 and PAI-2 was performed. PAI-2 but not PAI-1 mRNA was detected in fetal and neonatal epidermis, localized in the spinous layers. Although mRNAs for both inhibitors were induced by *tape*-*stripping* or hair-plucking, their distribution was more focal and more transient than that of uPA mRNA. These findings show that uPA, but not its usual inhibitors, is consistently elevated in the proliferative population of keratinocytes in a diverse range of hyperproliferative states. Two hypotheses are suggested by these data: (a) uPA may play a regulatory role in the activation of epidermal proliferation; or (b) uPA may be involved in the vertical migration of keratinocytes that must accompany increased cell proliferation.

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; Cell Division--physiology--PH; Epidermis--chemistry--CH; Epidermis --enzymology--EN; In Situ Hybridization; Keratinocytes--chemistry--CH; Keratinocytes--enzymology--EN; Mice; Mice, Inbred SENCAR; *RNA* , Messenger--metabolism--ME; Up-Regulation--physiology--PH
Chemical Name: *RNA*, Messenger; Plasminogen Activators

3/3,AB,KWIC/8
DIALOG(R)File 155:MEDLINE(R)

09153693 97038945 PMID: 8884526

ICAM-1 expression on keratinocytes in mechanically-injured skin of a patient with atopic dermatitis.

Matsunaga T; Katayama I; Yokozeki H; Nishioka K
Department of Dermatology, Tokyo Medical and Dental University, Japan. Journal of dermatological science (IRELAND) Sep 1996, 12 (3) p219-26 , ISSN 0923-1811 Journal Code: 9011485
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The expression of intercellular adhesion molecule-1 (ICAM-1) on keratinocytes was studied in skin specimens of patch- or scratch-test using Dermatophagoides farinae (DF) antigen in atopic dermatitis (AD). Positive patch test reaction showed exocytosis of lymphocytes and focal expression of ICAM-1 on keratinocytes. Similar lymphocyte infiltration and keratinocyte expression of ICAM-1 were observed in *tape*-*stripped* skin which was subjected as the control. In addition, focal ICAM-1 expression on keratinocytes was also observed without exocytosis of lymphocytes. By scratch-test with DF antigen, ICAM-1 expression on keratinocytes was observed in eight out of eleven patients. One specimen showed ICAM-1 expression in spite of the absence of exocytosis of lymphocytes. Two out of three specimens from negative scratch-test sites with control liquid (mixture of equal amount of saline and glycerine) showed ICAM-1 expression in a similar manner to that of the positive scratch tests. In normal control skin, patch-test sites showed focal and weak expression of ICAM-1. Normal appearing skin of AD also showed no ICAM-1 expression. Therefore, in patients with atopic dermatitis, keratinocytes may express ICAM-1 prior to infiltration of lymphocytes into the epidermis and this expression might be induced not only by cytokines produced by lymphocytes but also by mechanically injured (*tape*- *stripped* or scratched) epidermal cells.

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... to infiltration of lymphocytes into the epidermis and this expression might be induced not only by cytokines produced by lymphocytes but also by mechanically injured (*tape*-*stripped* or scratched) epidermal cells.

...; AD; Glycoproteins--immunology--IM; Immunohistochemistry; In Situ Hybridization; Intercellular Adhesion Molecule-1--genetics--GE; Keratinocytes--metabolism--ME; Keratinocytes--pathology--PA; Mites --immunology--IM; Physical Stimulation; *RNA*, Messenger--genetics--GE; *RNA*, Messenger--metabolism--ME; Skin--immunology--IM; Skin --injuries--IN; Skin--pathology--PA; Skin Tests

Chemical Name: Antigens; Dermatophagoides allergens; Glycoproteins; *RNA*, Messenger; Intercellular Adhesion Molecule-1

3/3,AB,KWIC/9
DIALOG(R)File 155:MEDLINE(R)

08877793 96243207 PMID: 8648167

Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis.

Wood L C; Elias P M; Calhoun C; Tsai J C; Grunfeld C; Feingold K R Dermatology and Medical Services, Veterans Administration Medical Center, University of California, San Francisco, USA.

Journal of investigative dermatology (UNITED STATES) Mar 1996, 106 (3) p397-403, ISSN 0022-202X Journal Code: 0426720

Contract/Grant No.: AR 19098; AR; NIAMS; AR 39448; AR; NIAMS; AR 39639; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Previous studies have shown that barrier disruption increases epidermal mRNA levels of interleukin-1 alpha (IL-1 alpha). We used immunohistochemistry to examine IL-1 alpha expression in hairless mouse skin under basal conditions and following barrier abrogation. In untreated mice, IL-1 alpha was present in the dermis and nucleated epidermal layers in a diffuse, generalized pattern. In essential fatty acid deficient mice IL-1 alpha was present in all epidermal layers and the dermis, with prominent staining in the stratum corneum. After acute barrier disruption with *tape*-*stripping*, IL-1 alpha increased in the epidermis and dermis within 10 min, remained elevated at 2 and 4 h, and decreased to near basal levels by 24 h. Moreover, intense, perinuclear, basal cell staining appeared at 10 min, persisting until 4 h after barrier disruption. Since the increase in IL-1 alpha immunostaining after acute barrier abrogation precedes the increase in mRNA, we hypothesized that the IL-1 alpha might derive from a pre-formed pool. Prolonged occlusion of normal skin, a treatment that specifically reduces epidermal mRNA levels of IL-1 alpha, decreased basal immunostaining for IL-1 alpha and blunted the increase in IL-1 alpha usually seen following barrier disruption. Moreover, *tape*- *stripping* of skin, maintained ex vivo at 4 degrees C, resulted in increased IL-1 alpha immunostaining within the upper nucleated epidermal layers, as well as release of mature IL-1 alpha into the medium, as measured by Western blotting and enzyme-linked immunosorbent assay. In addition, the stratum corneum attached to the tape contained IL-1 alpha. These studies show that acute barrier disruption induces both the immediate release and dispersion of IL-1 alpha from a pre-formed, epidermal pool, as well as increased IL-1 alpha synthesis; both mechanisms are consistent with a role for IL-1 alpha in the regulation of proinflammatory and homeostatic processes in the skin.

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...of IL-1 alpha, decreased basal immunostaining for IL-1 alpha and blunted the increase in IL-1 alpha usually seen following barrier disruption. Moreover, *tape*-*stripping* of skin, maintained ex vivo at 4 degrees C, resulted in increased IL-1 alpha immunostaining within the upper nucleated epidermal layers, as well as...

...; Epidermis--metabolism--ME; Epidermis--secretion--SE; Fatty Acids, Nonesterified--metabolism--ME; Gene Expression; Immunohistochemistry; Interleukin-1--secretion--SE; Mice; Mice, Inbred HRS; Occlusive Dressings; Permeability; *RNA*, Messenger--genetics--GE; *RNA*, Messenger --metabolism--ME; Skin--injuries--IN; Skin--secretion--SE Chemical Name: Fatty Acids, Nonesterified; Interleukin-1; *RNA*, Messenger

3/3,AB,KWIC/10
DIALOG(R)File 155:MEDLINE(R)

08603972 95363242 PMID: 7636313

Up-regulation of p21WAF1/CIP1 in psoriasis and after the application of irritants and *tape* *stripping*.

Healy E; Reynolds N J; Smith M D; Harrison D; Doherty E; Campbell C; Rees J L

Department of Dermatology, University of Newcastle upon Tyne, United Kingdom.

Journal of investigative dermatology (UNITED STATES) Aug 1995, 105 (2) p274-9, ISSN 0022-202X Journal Code: 0426720

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

p21WAF1/CIP1 is a nucleoprotein that was initially characterized by its ability to be regulated transcriptionally by p53 and by its ability to mediate growth arrest by binding to cyclin-dependent kinases. Although p21WAF1/CIP1 is thought to mediate the effects of p53 in causing growth arrest, p21WAF1/CIP1 is also regulated in a p53-independent manner, e.g., during terminal differentiation of some cell lines. Growth factors including epidermal growth factor also induce p21WAF1/CIP1 through p53-independent pathways. Because the epidermal growth factor signaling pathway is abnormal in psoriatic epidermis, we studied p21WAF1/CIP1 expression, using in situ hybridization and immunohistochemistry, in psoriasis. Both p21WAF1/CIP1 mRNA and protein were significantly elevated in untreated psoriatic plaques compared with uninvolved psoriatic skin ($p < 0.0001$), with the up-regulation of p21WAF1/CIP1 being predominantly suprabasal. This increase was accompanied by a small increase in p53 protein expression of uncertain significance. Furthermore, p21WAF1/CIP1 expression was induced in skin after sellotape stripping and by the application of agents, such as dithranol, that are capable of inducing hyperproliferation. The pattern of p21WAF1/CIP1 expression observed is consistent with a role in induction and maintenance of differentiation. Our experiments, however, cannot determine whether the abnormalities of p21WAF1/CIP1 epidermal expression in psoriasis and after insult are independent of changes in p53 expression.

Up-regulation of p21WAF1/CIP1 in psoriasis and after the application of irritants and *tape* *stripping*.

; Anthralin--pharmacology--PD; Cyclins--genetics--GE; Physical Stimulation; Protein p53--genetics--GE; Protein p53--metabolism--ME; Psoriasis--pathology--PA; *RNA*, Messenger--metabolism--ME; Sodium Dodecyl Sulfate--pharmacology--PD

Chemical Name: Cip1 protein; Cyclins; Irritants; Protein p53; *RNA*, Messenger; Sodium Dodecyl Sulfate; Anthralin

3/3,AB,KWIC/11
DIALOG(R)File 155:MEDLINE(R)

08336637 95096515 PMID: 7798624

Occlusion lowers cytokine mRNA levels in essential fatty acid-deficient and normal mouse epidermis, but not after acute barrier disruption. Wood L C; Elias P M; Sequeira-Martin S M; Grunfeld C; Feingold K R Dermatology and Medical Services, Department of Veterans Affairs Medical Center, San Francisco, CA 94121.

Journal of investigative dermatology (UNITED STATES) Dec 1994, 103 (6) p834-8, ISSN 0022-202X Journal Code: 0426720

Contract/Grant No.: AR 19098; AR; NIAMS; AR 39448; AR; NIAMS; AR 39639; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Acute disruption of the permeability barrier by either *tape* *stripping* or acetone treatment and chronic disruption by feeding an essential fatty acid-deficient diet increase the mRNA levels of tumor necrosis factor-alpha (TNF alpha), interleukin (IL)-1 alpha, IL-1 beta, IL-1ra, and granulocyte/macrophage-colony-stimulating factor in murine epidermis. Furthermore, epidermal TNF alpha protein levels also are stimulated by barrier disruption. To understand the relation of epidermal cytokine production to barrier function, we studied the effect of the application of a water vapor-impermeable membrane on epidermal cytokine production both in normal epidermis and after barrier disruption. Latex occlusion of essential fatty acid-deficient mice for 24-48 h lowered the mRNA levels of epidermal TNF alpha, IL-1 alpha, and IL-1ra to nearly control values, but not the levels of IL-1 beta mRNA. Occlusion of normal mice for 8, 24, and 48 h did not alter the levels of epidermal mRNAs encoding TNF alpha, IL-1 beta, or IL-1ra. Yet mRNA levels of IL-1 alpha, the major constitutively produced epidermal cytokine, were reduced by 40% after 24 h and by 80% after 48 h of occlusion of normal mouse epidermis. In contrast, latex occlusion of mice immediately after acute barrier disruption by either *tape* *stripping* or acetone treatment blocked neither the stimulation of epidermal mRNAs for TNF alpha, IL-1 alpha, IL-1 beta, or IL-1ra, nor the increase in epidermal TNF alpha protein. Taken together, these results suggest that barrier status regulates the production of specific cytokines in essential fatty acid-deficient and normal mouse epidermis. However, the signals that regulate epidermal cytokine production in response to acute barrier disruption do not appear to be influenced by occlusion.

Acute disruption of the permeability barrier by either *tape* *stripping* or acetone treatment and chronic disruption by feeding an essential fatty acid-deficient diet increase the mRNA levels of tumor necrosis factor-alpha (TNF alpha...

... and by 80% after 48 h of occlusion of normal mouse epidermis. In contrast, latex occlusion of mice immediately after acute barrier disruption by either *tape* *stripping* or acetone treatment blocked neither the stimulation of epidermal mRNAs for TNF alpha, IL-1 alpha, IL-1 beta, or IL-1ra, nor the increase...

; Cell Membrane Permeability--drug effects--DE; Cell Membrane Permeability--physiology--PH;

Interleukin-1--genetics--GE; Mice; Mice, Inbred HRS; *RNA*, Messenger--analysis--AN; Receptors, Interleukin-1 --antagonists and inhibitors--AI; Sialoglycoproteins--genetics--GE; Skin --physiopathology--PP; Tumor Necrosis Factor--genetics--GE Chemical Name: Cytokines; Fatty Acids, Essential; Interleukin-1; *RNA*, Messenger; Receptors, Interleukin-1; Sialoglycoproteins; Tumor Necrosis Factor; interleukin 1 receptor antagonist protein

3/3,AB,KWIC/12
DIALOG(R)File 155:MEDLINE(R)

08244937 95005886 PMID: 7921651
Barrier function coordinately regulates epidermal IL-1 and IL-1 receptor antagonist mRNA levels.
Wood L C; Feingold K R; Sequeira-Martin S M; Elias P M; Grunfeld C Dermatology Service, Veterans Administration Medical Center, San Francisco, CA 94121.
Experimental dermatology (DENMARK) Apr 1994, 3 (2) p56-60, ISSN 0906-6705 Journal Code: 9301549
Contract/Grant No.: AR 19098; AR; NIAMS; AR 39448; AR; NIAMS; AR 39639; AR; NIAMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Disruption of the cutaneous permeability barrier increases mRNA levels for TNF, GM-CSF, IL-1 alpha, and IL-1 beta in the epidermis. We have hypothesized that the cytokines mediate the changes in lipid and DNA synthesis which occur following barrier disruption. To further characterize the cytokine response to barrier abrogation, we examined the levels of epidermal IL-1ra mRNA in two acute models and one chronic model in the hairless mouse. IL-1ra mRNA levels increased shortly after acute disruption of the barrier with acetone, reached a peak at 3-4 h after treatment, and returned to control levels by 8h. These changes in mRNA levels parallel those which occur for IL-1 alpha and beta. Furthermore, IL-1ra mRNA levels were elevated 5-fold and 4-fold, at 2.5 h and 4 h, respectively, following *tape*-*stripping* , a second acute model of barrier disruption. Finally, IL-1ra mRNA levels were elevated 2.5-fold in the epidermis of EFAD mice, which have a chronic barrier defect. Thus, the cutaneous response to barrier disruption includes mechanisms which increase IL-1 and IL-1ra mRNA levels in a coordinate manner. The net result provides a regulatory mechanism for controlling the biological effects of increased IL-1 production.

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Descriptors: Epidermis--chemistry--CH; *Epidermis--metabolism--ME; * *RNA*, Messenger--analysis--AN; *Sialoglycoproteins--genetics--GE; Blotting, Northern; Densitometry; Epidermis--physiology--PH; Interleukin-1 --analysis--AN; Interleukin-1--genetics--GE; Interleukin-1--metabolism --ME; Mice; Mice, Inbred HRS; Permeability; *RNA*, Messenger--genetics --GE; *RNA*, Messenger--metabolism--ME; Sialoglycoproteins--analysis --AN; Sialoglycoproteins--metabolism--ME; Time Factors
Chemical Name: Interleukin-1; *RNA*, Messenger; Sialoglycoproteins; interleukin 1 receptor antagonist protein

3/3,AB,KWIC/13
DIALOG(R)File 155:MEDLINE(R)

08203048 94338172 PMID: 8060154
Permeability barrier disruption alters the localization and expression of TNF alpha/protein in the epidermis.
Tsai J C; Feingold K R; Crumrine D; Wood L C; Grunfeld C; Elias P M Dermatology Services, Veterans Administration Medical Center, University of California, San Francisco.
Archives of dermatological research (GERMANY) 1994, 286 (5) p242-8, ISSN 0340-3696 Journal Code: 8000462
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Previous studies have shown that (1) epidermal TNF alpha mRNA levels are increased following acute disruption of the cutaneous permeability barrier; (2) this increase is maximal at 1 h and decreases to control levels by 8 h; and (3) in essential fatty acid-deficient (EFAD) mice, a chronic model of barrier perturbation, TNF alpha mRNA levels are also elevated several-fold over controls. In the present study we determined, using immunocytochemical procedures, epidermal TNF alpha protein levels following either acute of chronic barrier disruption and the localization of any increase. Frozen, paraffin and Antibed sections of skin were incubated with polyclonal anti-mouse TNF alpha antisera and detection was accomplished by either immunoperoxidase or fluorescence procedures. We found that (1) TNF alpha-immunoreactive protein was present in normal mouse epidermis, and was primarily localized to the upper nucleated layers where it displayed a diffuse cytosolic pattern; (2) acute disruption of the barrier with acetone or *tape*-*stripping* resulted in TNF alpha staining that was more intense throughout all of the nucleated epidermal cell layers in comparison with normal epidermis; (3) the increase in TNF alpha staining occurred

as early as 2 h after barrier disruption; and (4) increased TNF alpha staining was also observed in the stratum corneum of EFAD mice. These results indicate that epidermal TNF alpha protein levels increase after both acute and chronic barrier disruption, and are consistent with the hypothesis that TNF alpha may signal and/or coordinate portions of the cutaneous response to barrier disruption.

... and was primarily localized to the upper nucleated layers where it displayed a diffuse cytosolic pattern; (2) acute disruption of the barrier with acetone or *tape*-*stripping* resulted in TNF alpha staining that was more intense throughout all of the nucleated epidermal cell layers in comparison with normal epidermis; (3) the increase...

; Blotting, Western; Cell Membrane Permeability; Epidermis--physiology --PH; Immunohistochemistry; Mice; Mice, Inbred HRS; *RNA*, Messenger --metabolism--ME

Chemical Name: *RNA*, Messenger; Tumor Necrosis Factor

3/3,AB,KWIC/14
DIALOG(R)File 155:MEDLINE(R)

08086878 94209449 PMID: 7512582

Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin.

Nickoloff B J; Naidu Y

Department of Pathology, University of Michigan Medical School, Ann Arbor.

Journal of the American Academy of Dermatology (UNITED STATES) Apr 1994 , 30 (4) p535-46, ISSN 0190-9622 Journal Code: 7907132 Contract/Grant No.: AR01823; AR; NIAMS; AR40065; AR; NIAMS; AR40488; AR; NIAMS

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND: An important function of skin is to serve as a barrier and thus provide protection from the external environment. The epidermal keratinocyte establishes this barrier by producing an intact stratum corneum. In the past, keratinocytes were appreciated only for this rather inert, passive structural responsibility and not for their potential dynamic contribution to inflammatory or immune-mediated reactions. OBJECTIVE: Our purpose was to examine the cascade of molecular and cellular events that occur when the barrier function of human skin is abrogated by repeated *tape* *stripping*, which physically removes the stratum corneum without inducing any cytopathic effects on the underlying epidermal keratinocytes. METHODS: Eight healthy human volunteers underwent repeated *tape* *stripping* and sequential punch biopsy specimens of skin obtained between 1 and 24 hours after *tape* *stripping* were analyzed for protein antigens by immunostaining of cryostat-cut sections. The presence or absence of various messenger RNAs (mRNAs) were detected by polymerase chain reaction. RESULTS: After repeated *tape* *stripping*, keratinocytes became activated within hours. The responses included up-regulation of keratin-16 expression and keratinocyte proliferation accompanied by production of a specific profile of cytokine and adhesion molecule mRNAs and proteins in both epidermal and dermal compartments. Polymerase chain reaction amplification of *RNA* species isolated from the epidermal portion of skin revealed increases 6 hours after *tape* *stripping* in mRNA coding for tumor necrosis factor-alpha, IL-8, IL-10, interferon gamma, intercellular adhesion molecule-1, transforming growth factor-alpha, and transforming growth factor-beta. There was no increase in tumor necrosis factor-alpha, IL-8, IL-10, or transforming growth factor-alpha mRNAs in the dermal samples. Immunostaining revealed that keratinocyte intercellular adhesion molecule-1 was increased 6 hours after stripping and was accompanied by endothelial cell expression of E-selectin (endothelial cell adhesion molecule-1) and vascular cell adhesion molecule-1. These molecular events, which occurred after 6 hours in *tape*-*stripped* skin, preceded any movement of inflammatory cells from the circulation into dermis or epidermis and hence reflect changes that occur in cells indigenous to normal human skin. None of these changes occurred in persons who underwent limited *tape* *strippings* without barrier perturbation. CONCLUSION: The results highlight the rapid and distinctive responses of epidermal keratinocytes and demonstrate that these cells can actively participate in a far greater number of homeostatic responses other than the production of the epidermal barrier.

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RNA species isolated from the epidermal portion of skin revealed increases 6 hours after *tape* *stripping* in mRNA coding for tumor necrosis factor-alpha, IL-8, IL-10, interferon gamma, intercellular adhesion molecule-1, transforming growth factor-alpha, and transforming growth...
...cell expression of E-selectin (endothelial cell adhesion molecule-1) and vascular cell adhesion molecule-1. These molecular events, which occurred after 6 hours in *tape*-*stripped* skin, preceded any movement of inflammatory cells from the circulation into dermis or epidermis and hence reflect changes that occur in cells indigenous to normal human skin. None of these changes occurred in persons who underwent limited *tape* *strippings* without barrier perturbation. CONCLUSION: The results highlight the rapid and distinctive responses of epidermal keratinocytes and demonstrate that these cells can actively participate in a... ...; Epidermis--metabolism--ME; Interferon Type II--biosynthesis--BI; Interleukin-10--biosynthesis--BI; Interleukin-8--biosynthesis--BI; Keratino cytes--cytology--CY; Molecular Sequence Data; Polymerase Chain Reaction; *RNA*, Messenger--analysis--AN; Skin--cytology--CY; Skin--immunology --IM; Transforming Growth Factor alpha--biosynthesis--BI; Transforming Growth Factor beta--biosynthesis--BI; Tumor Necrosis Factor--biosynthesis ...
Chemical Name: Cell Adhesion Molecules; Cytokines; E-Selectin; Interleukin-8; *RNA*, Messenger; Transforming Growth Factor alpha; Transforming Growth Factor beta; Tumor Necrosis Factor; Vascular Cell Adhesion Molecule-1; Interleukin-10; Interferon Type II

3/3,AB,KWIC/15
DIALOG(R)File 155:MEDLINE(R)

07490655 93018530 PMID: 1402399
Effect of cutaneous permeability barrier disruption on HMG-CoA reductase, LDL receptor, and apolipoprotein E mRNA levels in the epidermis of hairless mice.
Jackson S M; Wood L C; Lauer S; Taylor J M; Cooper A D; Elias P M; Feingold K R
Dermatology Service, Department of Veterans Affairs Medical Center, San Francisco, CA 94121.
Journal of lipid research (UNITED STATES) Sep 1992, 33 (9) p1307-14, ISSN 0022-2275 Journal Code: 0376606
Contract/Grant No.: AR-19098; AR; NIAMS; AR-39639; AR; NIAMS; DK-36659; DK; NIDDK; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Disruption of the permeability barrier results in an increase in cholesterol synthesis in the epidermis. Inhibition of cholesterol synthesis impairs the repair and maintenance of barrier function. The increase in epidermal cholesterol synthesis after barrier disruption is due to an increase in the activity of epidermal HMG-CoA (3-hydroxy-3-methylglutaryl CoA) reductase. To determine the mechanism for this increase in enzyme activity, in the present study we have shown by Western blot analysis that there is a 1.5-fold increase in the mass of HMG-CoA reductase after acute disruption of the barrier with acetone. In a chronic model of barrier disruption, essential fatty acid deficiency, there is a 3-fold increase in the mass of HMG-CoA reductase. Northern blot analysis demonstrated that after acute barrier disruption with acetone or *tape*-*stripping*, epidermal HMG-CoA reductase mRNA levels are increased. In essential fatty acid deficiency, epidermal HMG-CoA reductase mRNA levels are increased 3-fold. Thus, both acute and chronic barrier disruption result in increases in epidermal HMG-CoA reductase mRNA levels which could account for the increase in HMG-CoA reductase mass and activity. Additionally, both acute and chronic barrier disruption increase the number of low density lipoprotein (LDL) receptors and LDL receptor mRNA levels in the epidermis. Moreover, epidermal apolipoprotein E mRNA levels are increased by both acute and chronic perturbations in the barrier. Increases in these proteins in response to barrier disruption may allow for increased lipid synthesis and transport between cells and facilitate barrier repair.
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Descriptors: Acetone--pharmacology--PD; *Apolipoproteins E--genetics--GE; *Epidermis--metabolism--ME; *Hydroxymethylglutaryl CoA Reductases--genetics --GE; **RNA*, Messenger--metabolism--ME; *Receptors, LDL--genetics--GE Chemical Name: Apolipoproteins E; *RNA*, Messenger; Receptors, LDL; Acetone; Hydroxymethylglutaryl CoA Reductases

3/3,AB,KWIC/16
DIALOG(R)File 155:MEDLINE(R)

07422089 92355783 PMID: 1644919
Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice.
Wood L C; Jackson S M; Elias P M; Grunfeld C; Feingold K R Dermatology Service, Department of Veterans Affairs Medical Center, San Francisco, CA 94121.
Journal of clinical investigation (UNITED STATES) Aug 1992, 90 (2) p482-7, ISSN 0021-9738 Journal

Code: 7802877
Contract/Grant No.: AR 19098; AR; NIAMS; AR 39639; AR; NIAMS Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The disruption of the cutaneous permeability barrier results in metabolic events that ultimately restore barrier function. These include increased epidermal sterol, fatty acid, and sphingolipid synthesis, as well as increased epidermal DNA synthesis. Because tumor necrosis factor (TNF) and other cytokines are known products of keratinocytes and have been shown to modulate lipid and DNA synthesis in other systems, their levels were examined in two acute models and one chronic model of barrier perturbation in hairless mice. Acute barrier disruption with acetone results in a 72% increase in epidermal TNF 2.5 h after treatment, as determined by Western blotting. Furthermore, epidermal TNF mRNA was elevated ninefold over controls 2.5 h after acetone treatment. This elevation in TNF mRNA was maximal at 1 h after acetone, and decreased to control levels by 8 h. After *tape* *stripping*, a second acute model of barrier disruption that avoids application of potentially toxic chemicals, TNF mRNA was elevated fivefold over controls at 2.5 h. Moreover, the mRNA levels for epidermal IL-1 alpha, IL-1 beta, and granulocyte macrophage-colony-stimulating factor (GM-CSF) also were elevated several-fold over controls, after either acetone treatment or *tape* *stripping* , but their kinetics differed. GM-CSF mRNA reached a maximal level at 1 h after acetone, while IL-1 alpha and IL-1 beta were maximal at 4 h after treatment. In contrast, mRNAs encoding IL-6 and IFN gamma were not detected either in control murine epidermis or in samples obtained at various times after *tape* *stripping* or acetone treatment. The relationship of the cytokine response to barrier function is further strengthened by results obtained in essential fatty acid deficient mice. In this chronic model of barrier perturbation mRNA levels for epidermal TNF, IL-1 alpha, IL-1 beta, and GM-CSF were each elevated several-fold over controls. These results suggest that epidermal cytokine production is increased after barrier disruption and may play a role in restoring the cutaneous permeability barrier. ...h after acetone treatment. This elevation in TNF mRNA was maximal at 1 h after acetone, and decreased to control levels by 8 h. After *tape* *stripping* , a second acute model of barrier disruption that avoids application of potentially toxic chemicals, TNF mRNA was elevated fivefold over controls at 2.5 h...

...1 alpha, IL-1 beta, and granulocyte macrophage-colony-stimulating factor (GM-CSF) also were elevated several-fold over controls, after either acetone treatment or *tape* *stripping* , but their kinetics differed. GM-CSF mRNA reached a maximal level at 1 h after acetone, while IL-1 alpha and IL-1 beta were...

... In contrast, mRNAs encoding IL-6 and IFN gamma were not detected either in control murine epidermis or in samples obtained at various times after *tape* *stripping* or acetone treatment. The relationship of the cytokine response to barrier function is further strengthened by results obtained in essential fatty acid deficient mice. In...

; Cytokines--genetics--GE; Gene Expression; Granulocyte-Macrophage Colony-Stimulating Factor--genetics--GE; Interleukin-1--genetics--GE; Mice; *RNA*, Messenger--genetics--GE; Tumor Necrosis Factor--genetics--GE; Tumor Necrosis Factor--metabolism--ME
Chemical Name: Cytokines; Interleukin-1; *RNA*, Messenger; Tumor Necrosis Factor; Granulocyte-Macrophage Colony-Stimulating Factor

3/3,AB,KWIC/17
DIALOG(R)File 155:MEDLINE(R)

06769473 91083367 PMID: 2175580
Expression of guanine nucleotide binding proteins, Gs and Gi, in mRNAs in epidermal keratinocytes.
Takahashi H; Miyokawa N; Katagiri M; Iizuka H
Department of Dermatology, Asahikawa Medical College, Japan. Archives of dermatological research (GERMANY) 1990, 282 (6) p392-6, ISSN 0340-3696 Journal Code: 8000462
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The stimulation or inhibition of adenylate cyclase by hormones and chemicals is mediated by the stimulatory or inhibitory guanine nucleotide-binding proteins, Gs and Gi respectively. Although the presence of these G-proteins in the epidermis has been suggested, no direct information regarding their nature has been available. Using cDNAs for these G-proteins as a probe, we investigated the nature of the G-proteins of various keratinocytes. FRSK cells, a cell line derived from fetal rat epidermal cells, and SV-40-transformed human epidermal cells, both of which are highly proliferative keratinocytes, contained mRNAs for both Gs-alpha and two Gi-alpha proteins (Gi-2 alpha and -3 alpha). No evidence for the presence of Gi-1 alpha or Go-alpha was obtained. Normal human or pig epidermis had a relatively small amount of mRNAs for these G-proteins in the stable (hypoproliferative) condition. *Tape*-*stripping* -induced, and UVB-induced hyperproliferative epidermis contained an increased amount of these G-protein messages. However, all Gs-alpha, Gi-2 alpha and -3 alpha, as well as beta-actin mRNAs, were increased to a similar extent, and no specific expression of G-protein messages could be

detected in the hyperproliferative epidermis.

... Go-alpha was obtained. Normal human or pig epidermis had a relatively small amount of mRNAs for these G-proteins in the stable (hypoproliferative) condition. *Tape*-*stripping* -induced, and UVB-induced hyperproliferative epidermis contained an increased amount of these G-protein messages. However, all Gs-alpha, Gi-2 alpha and -3 alpha... Descriptors: GTP-Binding Proteins--genetics--GE; *Keratinocytes --metabolism--ME; **RNA*, Messenger--genetics--GE; Blotting, Northern; Cell Division--physiology--PH; Cell Line; Cell Line, Transformed; DNA Probes; GTP-Binding Proteins--metabolism--ME; Keratinocytes--cytology--CY; *RNA*, Messenger--metabolism--ME; Rats; Simian virus 40--physiology--PH ; Swine; Ultraviolet Rays
Chemical Name: DNA Probes; *RNA*, Messenger; GTP-Binding Proteins

3/3,AB,KWIC/18
DIALOG(R)File 155:MEDLINE(R)

05226249 86296545 PMID: 2427103
Onc-gene expression in hyperplasia induced by *tape* *stripping* or by topical application of TPA.
Giacomoni P U
British journal of dermatology (ENGLAND) Aug 1986, 115 Suppl 31 p128-32, ISSN 0007-0963 Journal Code: 0004041
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Guinea-pig ears were treated topically with 20 nmol of 12-O-tetradecanoyl phorbol-13-acetate, or deprived of their horny layer by nine successive strippings. At different times after the treatment, the animals were sacrificed, the epidermis removed from the ears, and the *RNA* from the epidermis purified and analysed by dot-blot hybridization in order to assess and determine the amount of *RNA* able to hybridize to each one of the 18 onc-gene DNA probes. The following probes were used: v-src, v-fps, v-yes, v-ros, v-myc, c-myc, v-erb AB, v-myb, v-mos, v-Ha-ras, v-Ki-ras, v-abl, v-fos, v-fes, v-fms, c-sis, B-lym, v-raf. At 0 h, expression of B-lym and of myc and fos is seen. ErbAB mRNA is detected between 10 min and 4 h after stripping, as well as after TPA application. B-lym mRNA is detected for up to 36 h after stripping and for up to 8 h after TPA application. C-myc mRNa is detected for up to 36 h after *tape* *stripping* , but only for the first hour after TPA application. *RNA* complementary to the other onc probes was not detected, and synthesis of *RNA* complementary to an actin DNA probe was observed for 8 h after TPA application.

Onc-gene expression in hyperplasia induced by *tape* *stripping* or by topical application of TPA.

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Chemical Name: Phorbols; Tetradecanoylphorbol Acetate; *RNA* ? logoff y
09dec02 12:56:14 User217743 Session D585.5
$2.82 0.880 DialUnits File155
$3.78 18 Type(s) in Format 4 (UDF)
$3.78 18 Types
$6.60 Estimated cost File155
$0.21 TELNET
$6.81 Estimated cost this search
$75.46 Estimated total session cost 5.617 DialUnits
Logoff: level 02.11.01 D 12:56:14